"NATURE". FEBRUARY 22, 1958
SUPPLEMENT: RECENT SCIENTIFIC AND TECHNICAL BOOKS

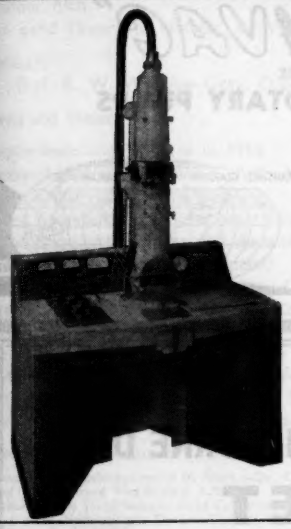
PRR

NATURE

A Weekly Journal of Science

UNIVERSITY OF MICHIGAN
PERIODICAL READING ROOM

Vol. 181, No. 4608 SATURDAY, FEBRUARY 22, 1958 2s. 6d.



AT YOUR SERVICE —

— a fully equipped electron microscope laboratory staffed by experienced operators

Write for details and charges to:—

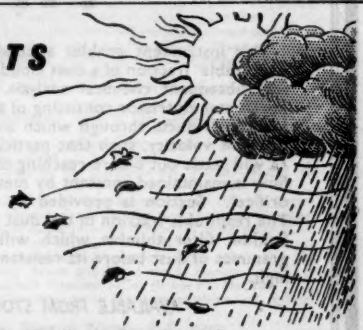
SCIENTIFIC APPARATUS DEPT.

METROPOLITAN-VICKERS
ELECTRICAL CO LTD · TRAFFORD PARK · MANCHESTER 17

An A.E.I. Company

N/R701

METEOROLOGICAL INSTRUMENTS



You will find the accuracy and robust construction you need in all meteorological instruments by Negretti & Zambra. Great sensitivity and long life are built-in qualities of every instrument we make.

Send for publication M/4, post free on request

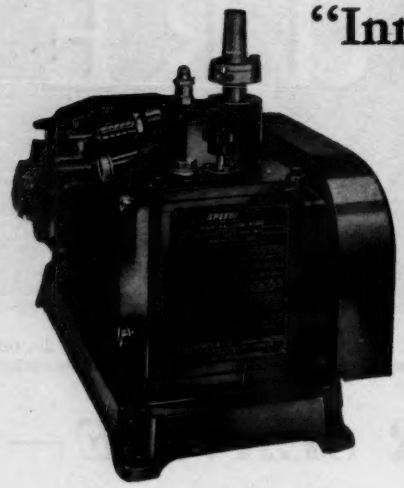
NEGRETTI & ZAMBRA
122 REGENT STREET · LONDON W1
Telegrams: Negretti, Piccy, London. REGent 3406

BRANCHES: BIRMINGHAM · CARDIFF · GLASGOW
LEEDS · MANCHESTER · NOTTINGHAM

TECHNICAL SERVICE AVAILABLE IN MOST COUNTRIES

ANEMOMETERS · BAROMETERS
HYGROMETERS · RAIN GAUGES
THERMOMETERS · SUNSHINE RECORDERS, etc.

Manufacturers of instruments for the indication, recording and controlling of temperature, pressure, liquid level, volume, specific gravity, humidity etc., aircraft instruments.



"Inner cleanliness comes first"

(with apologies to Messrs. ANDREWS Ltd.)

. . . and "SPEEDIVAC" ROTARY VACUUM PUMPS (2-stage series) ingeniously show the way through the exciting development of an integral OIL-RECIRCULATION SYSTEM which strips the operating fluid of all volatiles, permitting only the cleanest oil to re-enter the high vacuum stage—there's no risk of contamination impairing the highest vacuum operation.

AND, a word on quietness. "SPEEDIVAC" PUMPS are amongst the quietest pumps in the world—with or without AIR-BALLAST

"SPEEDIVAC"
TRADE MARK
HIGH VACUUM ROTARY PUMPS

For better vacuum service . . .

EDWARDS HIGH VACUUM LTD

MANOR ROYAL · CRAWLEY · SUSSEX CRAWLEY 1500 (10 LINES) EDCOHIVAC CRAWLEY

BRANCHES: GLASGOW · TORONTO & MILAN
AGENTS THROUGHOUT THE WORLD

CASELLA LONDON

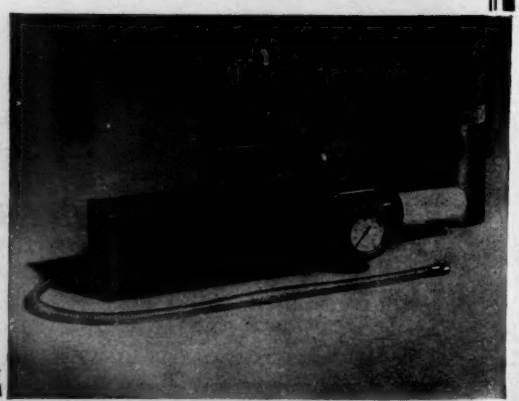
A NEW INSTRUMENT FOR SAMPLING AIRBORNE DUST—

THE HEXHLET

This instrument enables a bulk sample of the 'respirable' fraction of a dust cloud to be collected for subsequent chemical analysis. It employs a horizontal elutriator consisting of a number of flat horizontal ducts through which air is drawn at a constant velocity, such that particles above about 7μ will settle out before reaching the end. The air flow is maintained constant by means of a 'critical orifice'. Suction is provided by an air ejector. The respirable fraction of the dust is collected in a Soxhlet filter thimble which will hold up to 2 grammes of dust before its resistance becomes too high.

AVAILABLE FROM STOCK

Write for Leaflet No. 865



C. F. CASELLA & Co. Ltd
REGENT HOUSE, FITZROY SQUARE
LONDON, W.1
Telephone: ★EUSton 3944
Member Scientific Instrument Manufacturers' Association of Great Britain

NATURE

No. 4608 SATURDAY, FEBRUARY 22, 1958 Vol. 181

CONTENTS

	Page
Scientific Management in Nationalized Industries	511
Architect of British Medical Research. By Sir William Gavin, C.B.E.	513
Irrigation Works. By G. H. Lean	514
Diseases of Cereal Crops. By Prof. A. E. Muskett	515
Guides to Organic Chemistry. By Prof. John Read, F.R.S.	515
Electricity and Electrical Machines. By T. Wilson, D. A. Jones and Prof. G. W. Carter	516
Alpha-ray Activities of Humans and their Environment. By R. C. Turner, J. M. Radley and Prof. W. V. Mayneord	518
Formation of Two- and Three-Stranded Helical Molecules by Polyinosinic Acid and Polyadenylic Acid. By Dr. Alexander Rich	521
The Band Theory of Metals. By V. Heine	525
Obituary : Prof. R. W. Whytlaw-Gray, O.B.E., F.R.S. By Prof. Frederick Challenger	527
News and Views	528
Employment and Welfare in 1956	532
Deoxyribonucleic Acid and Genetic Modification in Ducks. By Dr. Alan W. Greenwood, C.B.E.	533
Union Observatory, Johannesburg	533
Forest Research and Development Expenditure in the United States	534
Photoperiodism in Plants and Animals. By Dr. P. F. Wareing	535
Farming History. By G. E. Fussell	536
Gelatine and Glue. By A. G. Ward	537
Pigmentation of Intestinal Muscle in Steatorrhœa. By Dr. G. Austin Gresham, J. G. Cruickshank and J. C. Valentine	538
Enzymic Synthesis of Coenzyme I in Relation to Chemical Control of Cell Growth. By Prof. R. K. Morton	540
A Remarkable Solar Radio Event. By Staff of the Ionosphere and Radio Astronomy Section of the Netherlands Telecommunications Services, The Hague	542
Solar Activity and the Ionosphere. By C. M. Minnis	543
Fossil Algae in India. By Prof. L. Rama Rao	544

Letters to the Editors :

Presence of Triiodothyronine in Fowl.—Dr. Claire J. Shellabarger and Dr. Rosalind Pitt-Rivers, F.R.S. 546
Enzymes of the Tricarboxylic Acid Cycle and Cytochrome Oxidase in the Fat Body of the Desert Locust.—Dr. D. A. H. Hearfield and Dr. B. A. Kilby 546
Phosphorylase Activity of Primary Rat Liver Cancer.—A. A. Hadjiolov and K. I. Dancheva 547
Occurrence of Independent Systems for the Cleavage of Folic Acid and Citrovorum Factor in Rat Liver.—U. W. Kenkare and Dr. Beatriz M. Braganca 548
A Possible Intermediate in the Biosynthesis of Tryptophan: 1-Deoxy-l-N-o-Carboxyphenyl-Ribulose.—Dr. F. W. E. Gibson, C. H. Doy and Shelagh B. Segall 549
Mode of Action of Chelates in protecting against *Clostridium perfringens* Toxin.—Prof. Merwin Moskowitz 550
Adherence and Viscosity of Blood Contacting Foreign Surfaces, and the Plasmatic Zone in Blood Circulation.—Dr. Alfred L. Copley 551
Isolation of Reserpine from *Vinca rosea* Linn.—Dr. N. K. Basu and B. Sarkar 552
Auxins in Immature Maize Kernels.—Dr. Kathleen R. Farrar, Joyce A. Bentley, G. Britton and S. Housley 553
Influence of Introduction of a Fructosyl Group into Galactosylglucoses on their β-Galactosidase-inducing Activity.—Gad Avigad 554
Demonstration of Some 'Masked' Lipids in the Oocytes of *Chrotogonus trachypterus* with Sudan Black *B* and Phenol 'Unmasking' Techniques.—Brij L. Gupta 555
Influence of Time on the Reversible Association between Large Molecules : the Collagen System.—Dr. Jerome Gross 556
Isomorphous Replacement in the Kaolin Group of Minerals.—Dr. R. F. Youell 557
Dynamics of Chain Molecules in Dilute Solutions, with Special Reference to Non-Newtonian Viscosity.—Dr. Roger Cerf 558
A Survey of Terrestrial Craters.—Dr. C. S. Beals, F.R.S. 559
Etch Figures on Sodium Chloride Crystals.—B. Jeszenszky 559
Relativistic Aberration Formula.—Dr. J. H. M. Whiteman 560
Random Time.—Prof. Norbert Wiener and Prof. Aurel Wintner 561
Contraction of Time and Protoplasm.—Prof. Anderson Nettleship 562
Propagation of Ultrasonic Waves in Liquids.—Dr. A. K. Dutta and K. Sama 563
Protection of Fœtuses against Radiation Injury.—Dr. E. M. Pantelouris 563
Hæmoglobin Types in Ox and Buffalo.—Dr. Francis Vella 564
Some Nutritional Requirements of *Colletotrichum coffeanum* Noack.—S. Bartniski-Garciá and Dr. C. Casas-Campillo 565
Nutritional Requirements of the Common Housefly, *Musca domestica vicina* Macq.—Prof. J. T. Chang and Miss M. Y. Wang 566
Occurrence and Filterability of Protoplast-like Elements in Aged Bacterial Cultures.—Dr. J. Sinkovics 566
Sexual Behaviour and the Vaginal Cycle in the Cat.—Richard P. Michael 567
Diseases of the Intervertebral Disk of the Cat.—A. S. King and R. N. Smith 568
Sudanophil Inclusions in Ameloblasts, Odontoblasts and Cells of the Oral Epithelium.—Prof. J. T. Irving 569
Effects of *Simulium* Control on Insectivorous Fishes.—Philip S. Corbet 570
Photosensitization of Bacteria by Furocoumarins and Related Compounds.—Dr. W. L. Fowlks, D. G. Griffith and Evelyn L. Oginsky 571
Cell Wall Composition in Bacterial and *L* Forms of *Proteus vulgaris*.—Dr. O. Kandler, A. Hund and C. Zehender 572
Antigenicity of a Bacterial Deoxyribonucleic Acid.—Dr. John H. Phillips, Prof. Werner Braun and Dr. Otto J. Plescia 574
Relaxation of Snails before Fixation.—Dr. Bruce M. McCraw 575
Occurrence of a 'Rare' Earthworm in Montgomeryshire.—K. Sylvia Richards 575
Three Zoopagales from Brackish Water.—F. R. Jones 575
Salmonellae in Uganda.—E. McAnulty 576
An Inexpensive Easily Constructed Fish-marking Tag.—Prof. Fergus J. O'Rourke and P. Makings 577
Chromosome Number of *Marsilea*.—Prof. P. N. Mehra and D. S. Loyal 577
Growth Responses of *Fusarium oxysporum* to Metabolites of Some Rhizospheric Microflora of Egyptian Cotton Varieties.—Dr. M. S. Naim and A. M. Hussein 578
A Simple Technique for producing Fruit Bodies of Wood-destroying Basidiomycetes.—N. Tamblyn and E. W. B. Da Costa 578
Comparative Chlorophyll Content of the Two Generations of Bryophytes.—Prof. Frederick T. Wolf 579
Propagation of Cacao in Plastic Bags.—R. Nichols 580

Recent Scientific and Technical Books Supp. viii

CHAPMAN & HALL

★ *Two John Wiley Books* ★

DISLOCATIONS AND MECHANICAL PROPERTIES OF CRYSTALS

Edited by

J. C. Fisher; W. G. Johnston; R. Thomson; T. Vreeland, Jr.

This work grew out of a small international conference, held in New York late in 1956, and sponsored by the Air Force Office of Scientific Research, Air Research and Development Command and the General Electric Research Laboratory. The book presents in a very complete manner the principal methods for observing individual dislocations. It reviews the parts of the field which are most complicated so that the present state of the subject is made quite clear.

Size: 9¼" × 6" *648 pages* *Illustrated* *120s. net*

HEAT TRANSFER

(Volume II)

by

Max Jakob

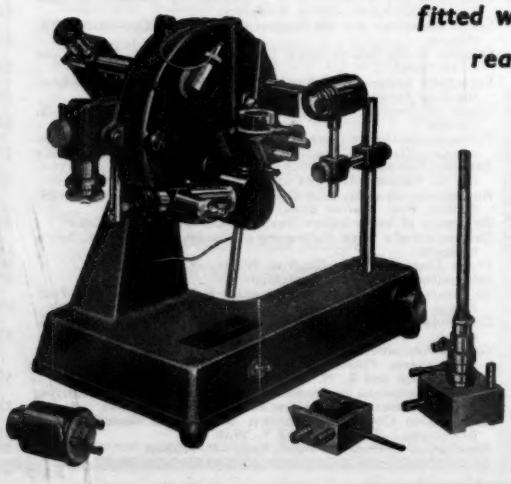
In the second volume of this classic work, the author deals mainly with selected fields of heat-transfer applications. He also offers a comprehensive analysis and discussion of heat radiation in spaces of simple configuration. A special feature is the inclusion of a section entitled "Supplements to Volume I", bringing up to date some of the material in that volume which needed additions or modifications as a result of later developments.

Size: 9¼" × 6" *684 pages* *Illustrated* *120s. net*

37 ESSEX STREET, LONDON, W.C.2

PRECISION BS INSTRUMENTS

Pulfrich Refractometer



fitted with circle divided on glass and giving readings on opposite sides of the circle to 5 seconds, by double microscope

This instrument possesses many advantages over the older type of Pulfrich refractometer and is used in all parts of the world for testing oils, optical glass, chemicals, drugs, etc.

We shall be pleased to demonstrate this instrument and give any advice as to its use, by appointment at our Works.

Bellingham & Stanley Ltd.

DEPT. N, 71 HORNSEY RISE, LONDON, N.19

PHONE: ARCHWAY 2270

SCIENTIFIC MANAGEMENT IN NATIONALIZED INDUSTRIES

LORD SIMON OF WYTHENSHAWE'S pamphlet, "The Boards of Nationalized Industries"*, arises out of his experience as governing director of two private engineering firms and as chairman of the British Broadcasting Corporation. It is a noteworthy contribution to the objective discussion of the nationalized industries which party politics have hitherto largely denied. The pamphlet expounds Lord Simon's conviction that the most important single reform in the nationalized industries is that the chairman and other members of the Board should have the same kind of experience, independence and security of tenure as is common in the best concerns of private enterprise; but it is of wider importance as endorsing the stress laid on the quality of management in the recent reports of the Fleck Committees dealing with the Atomic Energy Authority, to which reference has already been made in these columns (see *Nature*, February 15, p. 439).

The first of those reports stressed the over-riding importance of a suitable organization for recruitment, training and progression of promotion of technical staff and of a positive policy of selection of promising men at an early stage in their careers, followed by planned training on the job by moving them from one part of the organization to another. That policy should be designed to provide managerial staff with a sound understanding of all the main aspects of the organization's work, and while the report outlines in some detail general principles of organization which should govern a large technological production group, it repeatedly stresses the need for some scientific and technical knowledge on the part of those holding the highest managerial posts. The question of management is thus put right in the context of the shortage of scientific and technical man-power and of Britain's need to expand as a matter of urgency both technological and scientific education as well as to modify it to provide men of the qualities needed. The report of the second committee dealing with nuclear safety points no less emphatically to the shortage of technical staff in this field at all levels, though the emphasis is not so much on the management side.

Lord Simon comes out as emphatically as the well-known report of the Fleck Committee on the organization of the National Coal Board as to the vital importance of the quality of leadership at the highest level, and the need for encouraging the development of people capable of filling those positions. The most important single quality in the successful conduct of any large concern, whether public or private, is the quality of the highest authority in it, and Lord Simon's careful comparison of the board of Imperial Chemical Industries, Ltd., with the National Coal Board is intended to throw into relief the strength and weakness of the two boards and the way they are selected, and especially to suggest means by which the National Coal Board could be strengthened. One other observation is of interest in relation to the expansion of technological education, and in particular to the need for fuller attention to training for management. In spite of all the efforts of the National Coal Board in training managers, it must be expected, writes Lord Simon, that another ten or twenty years will elapse before there will be an adequate supply of well-trained executives ready to take on jobs as members of the Board.

This long-term aspect of the selection and training of managers gives urgency to the expansion of technological education in Britain, and to its modification so as to ensure that both the colleges of technology and the universities provide industry and the public services with a constant supply of men and women imaginatively alive to human issues as well as to the scientific and technical aspects of the problems which will confront them—both civilized scientists and citizens understanding the essentials of science, as Sir Hugh Beaver puts it. Unless that supply is ensured and appropriate measures are taken now, both as regards numbers and quality, men of the desired quality may not be forthcoming in sufficient numbers even if, as Lord Simon suggests, the nationalized industries follow the methods adopted by the leading concerns in private enterprise in recruiting their boards. Like the Windscale accident, this analysis of the experience of the National Coal Board points to the need for vision, foresight and vigilance on the part of all concerned.

Lord Simon's survey of private enterprise is not limited to Imperial Chemical Industries, Ltd., though he gives greater details of that firm's board and of the National Coal Board. He points out very fairly that the boards of most good private enterprises to-day, especially some of the largest, are marked by a very deep sense of accountability not only to their shareholders but also to their employees, to their customers, and to the community. There is little reason for thinking that the general attitude of their board members either to efficiency or to public service differs from that of the board members of a nationalized concern.

Reviewing the boards of eight large private enterprises, Lord Simon notes the considerable number of graduates among their members, the substantial number of full-time directors, the care taken in the selection of new directors, and the background of long experience in the industry possessed by the majority of directors, in addition to the usual qual-

* The Boards of Nationalized Industries. By Lord Simon of Wythenshawe. Pp. 54. (London: Longmans, Green and Co., Ltd., 1957.) 2s. 6d. net.

ities that are looked for in an administrator and director of policy. The outstanding characteristic stressed by Lord Simon is their security of tenure. Changes in the boards are slow, and while this encourages confidence in the security of their jobs on the part of the directors, the consequent continuity is of the first importance in building up good traditions, wise policy and high efficiency.

It is here that private industry is in most marked contrast with the nationalized industries. Whereas the director of a large private company is virtually sure of keeping his job until he reaches retiring age, the National Coal Board, for example, has been reconstituted twice since 1947. There have been twenty-four full-time members of the three Boards, although the number at any one time has never exceeded nine. The average length of service of those who have left has been 3·6 years, and no present member has yet served for three years; nevertheless, Lord Simon thinks that the present Board, whose members, on the average, have more experience of the industry than the average of the first two Boards, may be much more stable than its predecessors.

Lord Simon is convinced that security of tenure is by far the most important step towards continuity and efficiency of a board of directors, and it will be recalled that the Fleck Report on the National Coal Board also stressed the importance of continuity. Membership of the board of a nationalized industry should, he argues, be a career appointment; and although he agrees that the members should be appointed by the appropriate Minister, this should only be done after full consultation with the chairman and with the acceptance of the board. Further, candidates should have been selected and trained by the methods and on the scale of the best known practice, and appointments once made should continue up to retiring age, subject to the power of the Minister to terminate any appointment—a power which should rarely be used. Salaries, pensions and other conditions of service should be at least comparable with those in leading private firms; in this connexion the recent revision of salaries of members of various public boards, taken with the security of tenure stressed by Lord Simon, may help to overcome the present reluctance of outstanding men in a nationalized industry to accept appointments to its board.

This factor will obviously be of increasing importance when in ten to twenty years time the boards of the nationalized industries have an adequate supply of first-rate candidates from within the industry competing for vacant positions on them. Lord Simon's remarks on accountability also have a bearing in this connexion, though their chief interest is in a wider context. Having obtained a good board, an industry should be given the maximum freedom and responsibility subject to the necessary minimum of control by the Minister. This is essential if the board is to acquire the status that ultimately will enable it to recruit members of the calibre desired; and incidentally, as Lord Simon notes, to attract to the industry a sufficiency of the ablest graduates leaving the universities and colleges of technology of Great Britain.

The question of the exercise of Ministerial powers is regarded by Lord Simon as mainly a matter of good sense, but it should be a matter of high principle for every Government and every Minister to allow a board the maximum freedom and responsibility within the broad limits of national policy. On the strict issue of accountability, Lord Simon maintains that Parliament must realize that it is not competent to contribute anything whatever to the efficiency of, for example, the coal industry, while too much interference may cause serious inconvenience and even inefficiency. He agrees with Lord Heyworth that autonomy and responsibility to Parliament are inconsistent, and approves of Mr. Herbert Morrison's suggestion that accountability is best served by a periodical inquiry, at intervals of not less than seven years.

Lord Simon recognizes the value of parliamentary discussions in clarifying broad lines of public policy and indicating trends of public opinion, and also that the Parliamentary question, if it avoids matters of day-to-day management, is an important means of securing information and a safeguard against abuse. Here again it is largely a matter of common sense, and accountability may well become easier as the discussion of the problems of nationalized industries becomes more objective. That in itself should contribute to public confidence in the industries, as well as encourage greater stability and self-confidence within the industry and thus provide conditions in which the industry can develop its own standards and criteria of efficiency.

Here a suggestion made by Mr. R. Nottage, director of the Royal Institute of Public Administration, could be helpful. Discussing the use of the annual reports of the nationalized industries, he suggests that the adoption of a standard pattern might assist Parliamentary discussion. The record of operational results, both physical and financial, should facilitate adequate comparison with previous years and an understanding of the basis on which indices are selected. Presentation in such a form, with adequate reference to future plans and the periodical discussion of major topics on such a time scale as is adopted, for example, in the Unilever annual report, would give Parliament the information which the public interest rightly demands, and turn discussion into constructive channels rather than the detail and sterile issues which have been encouraged so much in the past. Lord Simon at least has indicated some of the opportunities. The juxtaposition of his pamphlet with reports of the Fleck Committees and of some other management literature should give a fresh impetus to measures for the expansion of scientific and technological education in Britain, upon the adequacy of which the quality of management and the efficiency of British industry ultimately depend. Education is, as always, the keystone of any such developments, and until a broad view is accepted of the scope of technical and technological education, the problem of supplying the men for the higher levels of management will remain with us.

ARCHITECT OF BRITISH MEDICAL RESEARCH

The Bright Countenance

A Personal Biography of Walter Morley Fletcher by Maisie Fletcher. Pp. 351+8 plates. (London: Hodder and Stoughton, Ltd., 1957.) 25*s*. net.

I HAVE heard it argued that it is a mistake for anyone to review the biography of a friend. He is biased from the start and likely to pay too much attention to the biographer's treatment of his friend, and too little to the literary or historical merit of the book itself. If this is so, then I should not be reviewing Sir Walter Fletcher's biography, for I am certainly biased from the start. Fifty years ago he was my tutor at Trinity College, Cambridge. He never attempted to preach at undergraduates or talk down to them. Instead he held up to us, quite unconsciously and in an almost diffident manner, a great example of poise, manliness and integrity of thought. Here was a man, we felt, firm of purpose and bound to the highest ideals of behaviour—a guide to be remembered in facing the moral and material issues of life which lay before us.

It was with these thoughts in mind that I opened this biography by his widow with some anxiety, for it is notoriously difficult for any woman to paint a true picture of her husband—difficult for her to interpret actions or even characteristics apart from their impact on her and apart from her own intimate association with them. I need not have feared. Lady Fletcher has succeeded in being objective. I am sorry, however, that, doubtless with this in view, she has written in the third person, thus removing not only herself but also the reader one stage from the scene. On the whole I should rather have read "I was delighted to see him back" than "Maisie was delighted to see him back". Another regret is that she has not used as her title Milton's words in full—"the bright countenance of truth". Not everyone is familiar with the quotation, and her publishers should not have allowed the abbreviation which makes the title so dreadfully misleading.

These, however, are small points. To me, at any rate, this book was a strangely moving revelation. His singleness of purpose, his amazing industry, his freedom from petty thoughts of all kinds, his joy in holidays and all out-door activities, his readiness to give always of his best whether in small things such as helping a troubled undergraduate or in great affairs of national medical policy—all these things we knew. What we now more fully realize is his courage, his deep spiritual convictions, and the real happiness he found in his family, his home and his friends.

To turn from the man to his work: this falls into two main periods—first, his years at Cambridge as research worker and tutor and Fellow of Trinity College, and then after 1914 his secretaryship of the Medical Research Committee (later Council) until his death in 1933.

His research work is summarized in Sir Arthur MacNalty's supplement to the book under review, but as full accounts are available elsewhere—notably in Prof. T. R. Elliott's obituary notice ("Obituary Notices of the Fellows of the Royal Society", **1**, 153; 1933)—there is no need to refer to it in any detail here. It is sufficient to say that his studies, extending over some sixteen years, on the chemistry of muscular respiration were classical. As W. B. Hardy wrote, "origins are as difficult in science as in literature: here, however, it is safe to credit Fletcher with that first step that counts". He gave a new direction to biological thought.

From 1914 onwards Fletcher's life-work was to originate, inspire and support the research work of others. The National Insurance Act of 1911 provided that for each insured person one penny should be set aside for medical research. This, it was thought, would yield £57,000 per annum—an interesting comparison with to-day's annual expenditure of some £2½ million. The original proposal was to assign the whole sum to research on tuberculosis, a disease which was expected to be especially costly to the taxpayer. Wiser counsels prevailed, however, thanks principally to Dr. Christopher Addison (Viscount Addison), Lord Astor and Sir Robert Morant, and it was finally enacted that research could cover "any disease to which insured persons are liable". A Committee under Lord Moulton of Bank was appointed to administer the fund, with an advisory council of some forty medical men. But the appointment of a secretary was long postponed. As Gowland Hopkins wrote, "an ideal appointment seemed almost impossible of achievement. Looking back to those weeks during which it was in suspense, I feel that the future of medical research in this country was then facing a critical moment. But at last came the happy solution and it was T. R. Elliott who suggested it". Fletcher's appointment met with instant and general approval, particularly from Sir Robert Morant, chairman of the National Insurance Committee to which the Medical Research Committee was attached, who was a man inspired by almost fanatical zeal for benefiting the people of England in education, health and well-being. "These ideals," wrote a friend, "found not an echo but a counterpart in what had been innate in Fletcher, whose spiritual depths were uplifted by the vision of service for others in so wide a field. Scientific research became more than a beautiful use of the intellect in approaching the truths of Nature, for he now realized the need by England of its direct application to all the problems of ill-health. Henceforward he, too, became a great public servant and sank his own conspicuous abilities for research in guiding and helping countless other men."

From the first the Committee's policy was to set up a comparatively small central research institute with its own staff, and to spend the greater part of the funds in support of investigations in private or voluntary institutions, where hitherto nearly all medical research had been done with little if any help from the State. The Mount Vernon Hospital at Hampstead was purchased and four Departments set up in temporary accommodation—(1) Bacteriology (Sir Almroth Wright and S. R. Douglas), (2) Biochemistry and Pharmacology (H. H. Dale), (3) Applied Physiology (Leonard Hill and Martin Flack), and (4) Statistics (J. Brownlee). A proposal to include a research hospital of twenty beds was abandoned on Fletcher's advice: he rightly considered that clinical research could better be carried out at existing and fully equipped hospitals.

The outbreak of the First World War interfered with many other plans, but Fletcher at once saw that military needs gave him an opportunity for establishing beyond all doubt the importance of medical research and the vital service which it could render.

He submitted a White Paper (Cd. 7922) containing proposals for research in direct connexion with the war and, most important of all, established full collaboration with the Army Medical Department. With the active help of Sir Alfred Keogh, Director General of the R.A.M.C., a channel for information and research on specific problems was operative between the Medical Research Committee and the army medical services in the field and home hospitals throughout the war.

With the return of peace and the establishment in 1919 of the new Ministry of Health came another moment of crisis in the history of the Medical Research Committee, for it was proposed to place it under the control of the new Ministry. Fortunately Fletcher had two enlightened men to deal with—Addison and Morant. Fletcher argued, first, that a progressive Ministry of Health will inevitably find itself committed from time to time to particular policies of health administration. A research organization under its control would be obliged to keep in step with current policy, instead of being free to recommend changes in policy in the light of new knowledge. Secondly, if the research organization formed part of the Ministry, its activities might come to be unduly restricted to problems of pressing practical urgency relating to the Ministry's executive functions, thereby excluding fundamental work which in the long run might be of more value. Thirdly, the Ministry operated only in England and Wales, but research organizations should cover the United Kingdom and reach out to the whole Commonwealth and Empire.

These views were accepted and in 1920 the Medical Research Committee became by Royal Charter the Medical Research Council, reporting to the Privy Council (an arrangement already in force with the Department of Scientific and Industrial Research and to be repeated in 1931 with the Agricultural Research Council). Only the other day a senior official of the Ministry of Health commented on this decision. "I am devoted", he said, "to the Ministry I have served so long. I believe it has done and is doing good work. But the greatest of the services which Fletcher rendered to medicine was to prevent the Medical Research Council from becoming its servant."

The subsequent progress and achievements of the Council need no recapitulation here. As Sir Arthur MacNalty writes, "it is not given to every pioneer to see the results of his labours, but Fletcher had the supreme satisfaction of knowing that the cause to which he had devoted all his energy and gifts had triumphed, and that he had established medical research in this country on secure foundations".

Lady Fletcher has written this biography primarily, she says, for "Walter's nine grandchildren", but she has placed all who knew him in her debt. Two comments which she quotes together sum up, I think, what we realize still more clearly after reading her book. The first is from *The Times* leader on the day after his death: "Fletcher was friend to every honest seeker after truth and he possessed in special degree the faculty for friendship. . . . His loyalty was unquenchable and he served no ends except those of the good of his fellows". The other is from his cousin, Sir Clement Jones: "Ever since my first year at Trinity I remember Walter as one who excelled in everything he tried to do, and he always aimed at the very best things and the high and honourable things. I shall never hear his name mentioned without thinking of those high standards in work, in play, in conversation and perhaps above all in friendship". May I, in conclusion, add one more quotation, from a letter by Sir Ernest Gowers, because it so exactly reflects my own feelings: "I never ceased to feel his friendship was an honour as well as a delight".

WILLIAM GAVIN

IRRIGATION WORKS

Irrigation and Hydraulic Design

By Dr. Serge Leliavsky. Vol. 2: Irrigation Works. Pp. xvi+864+85 plates. (London: Chapman and Hall, Ltd., 1957.) 294*s* net.

THIS, the second volume of Dr. Leliavsky's monumental trilogy on irrigation, is devoted to the specific design of irrigation works. The first volume, comprising Chapters 1–3, contained the theoretical background necessary for the hydraulic side of the design, while this volume, comprising Chapter 4–8, is concerned with the detail of the structures and irrigation planning. As in the first volume, the approach is basically historical, a treatment which has both advantages and disadvantages. The main advantage for the reader is that of having the steady progress of the subject explained and commented on by one who was himself in daily contact with, and in the forefront of, these developments. The disadvantage is that as a design manual, which is the intention of the book, the treatment in places becomes discursive and prolix.

The scope of the book is sufficiently well illustrated by the chapter headings, which are: the design of regulators (Chapter 4); the design of irrigation and drainage syphons (that is, cross-drainage works) (Chapter 5); weirs and other hydraulic works (Chapter 6); planning and designing a perennial irrigation scheme (Chapter 7); and canal bridges, aqueducts and gates (Chapter 8). The engineer will particularly welcome the detailed analyses of examples of various types of structure drawn from existing works mainly within the author's own experience. Study of these should enable a competent engineer to design similar structures for himself. The last chapter comprises more than a third of the book, and is devoted exclusively to structural design. In the case of gates, the author is here concerned with the static loading, although vibration normally plays a major part in failure, as it did in the one case quoted by him. In general, problems of chatter and fatigue failure loom large in high head structures, and designers would welcome more guidance on these matters.

The general system of irrigation used as a model in the book is naturally, in view of the author's close association with it, that practised in Egypt. It is clear that the main problem now facing engineers in that country is waterlogging, due to the too liberal application by cultivators who had for many years been starved of water. However, the problem of soil salinity now menacing the irrigation communities in India does not seem to have appeared in Egypt. A comparison of the two systems might reveal why this is so, and it might well be that the installation of a system of drains and pumping stations rendered necessary to deal with waterlogging in Egypt might also have to be faced in India for the quite different purpose of leaching out salts.

The second major difficulty of irrigation, namely, siltation and the problem of designing canals which neither silt nor scour, has received a somewhat superficial treatment, the author contenting himself with quoting a number of design formulæ and graphs without probing the matter more deeply. The reasons for the different formulæ used in Egypt and India are not stated and no guidance is given as to the particular form to be adopted in other areas. The most recent formula for the velocity–depth relation for non-silting canals has quite a different form from that previously adopted, although a thorough analysis is precluded by lack of data concerning the size and amount of solids carried by the canal.

The layout of the book is excellent and the text, which is both lucid and fluid, is illustrated by a large number of diagrams and plates. However, design offices will regret the frequent changes of units.

G. H. Lean

DISEASES OF CEREAL CROPS

Cereal Diseases in Ireland

By Prof. Robert McKay. Pp. x+161+54 plates. (Dublin: At the Sign of the Three Candles, 1957.) 21*s*.

THIS is the sixth in the series of handbooks dealing with crop diseases in Ireland written by Prof. R. McKay, illustrated with splendid photographs by G. H. McLean and published by the Three Candles Press. Of similar format to its predecessors, this volume deals with the diseases of cereal crops; it has an excellent frontispiece in colour with occasional colour plates scattered through the text. The foreword is by the Earl of Iveagh. The book is divided into six sections with an appendix. The sections deal respectively with the rusts, smuts, other parasitic diseases, parasitic diseases occasionally seen, miscellaneous diseases of non-parasitic origin and eelworm attack, while the appendix gives some account of the barberry and buckthorn, alternate hosts of some of the most important cereal rusts, with special reference to their distribution in Ireland. It could be suggested that the material dealt with in the appendix would have been better placed with the section dealing with the rusts.

Some passages, such as appear, for example, in the section dealing with loose smut of oats, read a little more like a text-book of plant pathology than a semi-popular treatise, but this merely illustrates the great difficulty the author of such a work as this has in consistently envisaging the needs of the readers for whom the book is intended. It is no easy matter to prepare a glossary of terms for a work such as this and, although a useful one is provided, the omission of some such as ascocarp, stroma, mesocotyl, etc., is noticeable. The illustrations of disease symptoms are profuse and excellent, but when so much mention is made in the text of the various spore forms of the rust fungi, for example, it seems a pity that some attempt was not made to illustrate these and their methods of germination. The choice of references would have been improved by being more inclusive.

The book is written in a simple straightforward style and is very readable. Part of the reason for its appearance is as a guide to agricultural advisers to help them to be able to identify, more readily, cereal diseases in the field. It is also intended to be of service to young farmers attending winter classes in agriculture or engaged in club work. The book is excellently produced and maintains the high standard of the Three Candles Press. There are very few errors, printing or otherwise, and only two were detected. The illustrations are particularly valuable and up to the high standard one might expect. Priced at one guinea, the publication is worth the money as a book, although it may seem a little expensive for many for whom it is intended. The whole tenor and tone of the work, like that of the earlier volumes, denote the enthusiasm of the author and mark him out as one of the keenest plantsmen that Ireland has produced.

A. E. Muskett

GUIDES TO ORGANIC CHEMISTRY

Organic Synthesis

By Dr. Vartkes Migrdichian. Vol. 1: Open-Chain Saturated Compounds. Pp. xxviii+834. Vol. 2: Open-Chain Unsaturated Compounds; Alicyclic Compounds; Aromatic Compounds. Pp. xiv+835–1822. (New York: Reinhold Publishing Corporation; London: Chapman and Hall, Ltd., 1957.) £14 the set of two volumes.

SINCE the inception of the fundamental theory of organic molecular structure a century ago (1858), with the addition seven years later of that "crowning achievement of the doctrine of the linking of carbon atoms", the idea of the benzene ring, organic chemistry has advanced in a kind of geometrical progression. A concrete expression of its vast scope at the present day is embodied in the current edition of "Beilsteins Handbuch", which includes to date some ninety massive volumes, although (except for the latest volume) these cover the literature only to 1929. It is understandable, therefore, that the specialist in organic chemistry is nowadays largely dependent upon various types of more concise and up-to-date guides to this great efflorescence of organic chemical information. Such guidance is afforded by a multiplicity of monographs on special fields and by various periodicals exemplified by the American publication, "Chemical Reviews", and the British "Annual Reports", *Quarterly Reviews* and *Current Chemical Papers*.

A fundamental problem lies in the effective presentation of the large number of chemical reactions now available for preparative and research work, in both pure and applied organic chemistry. Very helpful in this way are the American publications, "Organic Syntheses", an annual collection of reliable preparative methods for individual compounds, and "Organic Reactions": each of the nine volumes of the latter, issued since 1942, deals in detail with a number of reactions of synthetical importance.

Dr. Vartkes Migrdichian's work, although bearing upon the same ultimate problem as these two publications, differs from them in two important respects. First, it is not a periodical but a complete work; secondly, his review of reactions is comprehensive and systematic, instead of being limited to an arbitrary selection and arrangement. The first volume covers the open-chain saturated compounds; the second takes in the much wider field of open-chain unsaturated compounds, alicyclic compounds, and aromatic compounds. A third volume dealing with heterocyclic compounds is contemplated.

The thirty chapters follow closely the arrangement of a standard text-book of organic chemistry. Methods of formation are followed by a review of reactions of the type of compound under discussion. The Grignard and Friedel–Crafts reactions, and the diene synthesis, with their exceptionally wide applications, are treated separately. In general, however, reactions are classified under the heading of compounds of the type with which they are chiefly associated: thus, the Fries reaction will be found under the heading of phenol esters; and the Wagner, Nametkin and Demjanow rearrangements under alicyclic hydrocarbons.

The work is a useful compendium of an enormous amount of material, and is fully documented, although the literature of the past decade is sometimes sparsely represented. Theoretical discussions are not included, and reaction mechanisms are mentioned only when generally accepted. The text, formulæ and tables are clearly printed by a modern economic process; nevertheless, the price is distinctly high. As might be expected, in so extensive a compilation, misprints are not uncommon.

Dr. Migrdichian, who is senior research chemist to the American Cyanamid Co., with a record of some thirty years in the field of work leading to these volumes, is to be congratulated upon the result of prolonged labours that will minimize those of the many organic chemists who will derive welcome help from this publication.

JOHN READ

ELECTRICITY AND ELECTRICAL MACHINES

Electrical Engineering (General)

Electrical Circuits—Instruments and Measurements—Electric and Magnetic Fields—Conductors—Insulating and Magnetic Materials—Electronic Tubes. By A. T. Dover, Dr. F. T. Chapman, Prof. F. Brailsford and Harley Carter. (Electrical Engineering Series.) Pp. ix+489. (London and New York: Longmans, Green and Co., Ltd., 1956.) 50*s*. net.

Electrical Machines

By A. Draper. (Electrical Engineering Series.) Pp. xvi+346. (London and New York: Longmans, Green and Co., Ltd., 1956.) 40*s*. net.

The General Theory of Electrical Machines

By Bernard Adkins. Pp. ix+236. (London: Chapman and Hall, Ltd., 1957.) 45*s*. net.

THE first two books belong to a series intended for use by students preparing for Higher National Certificate, degree, and other examinations. The preface states that "Electrical Engineering (General)", the first of the series, is limited in scope to theory connected with electric circuits, instruments and materials for use with direct currents and alternating currents of power frequencies. The manner and depth of treatment vary considerably from topic to topic. Three chapters contributed by Prof. Brailsford provide a lucid and up-to-date account of ferromagnetic phenomena. Unfortunately, the same fundamental approach has not been applied to the discussion of conducting and insulating materials, the treatment of which is reminiscent of an engineer's handbook. This criticism could also be levelled at some of the material concerning commercial measuring instruments. It is perhaps inevitable that a book written by a panel of authors should consist of discrete sections, but it must be confusing to students to find, for example, a discussion of network theorems as part of direct-current circuit theory, without any mention of their general application, while later the star-delta equivalent impedances of a 3-phase circuit are derived without any reference to an earlier and similar derivation among the network theorems. Despite the declared limitation to power frequencies, two chapters on electronics describe various types of radio-frequency apparatus. A useful and comprehensive selection of examples, with answers, many taken from examination papers, is provided at the end of the book.

"'Electrical Machines' has been written as a text-book for students and not as a treatise for designers." Here, at last, students can find under one cover details of nearly all types of electrical machines. The omission of the single-phase machine is, however, to be regretted; it is, after all, very common. Academically there also appears to be no justification for including the mercury-arc rectifier in a book on machines. The approach to the subject is logical and on traditional lines, and should appeal to the student of electronics studying machines as a secondary subject. He will also find the sections on rotating and magnetic amplifiers very useful. The book can be recommended unconditionally to such students. Those taking heavy-current subjects for a degree course will probably wish for greater depth of treatment, but it is an excellent second-year book. The author and the British Thomson-Houston Co., Ltd., are to be highly commended for the clarity of the diagrams, so essential to a book on machines. The examples at the end of the book are well chosen, even though there is a mixture of units. The appendix on the measurement of three-phase power will prove very useful to students in the laboratory.

The general theory of electrical machines has been presented by several writers in a form so mathematical as to appear remote from whirling masses of copper and iron, and the publication of Mr. Adkins's book, in which the theory is applied to computing the details of machine performance under varying conditions, is all the more welcome. The author is a reader in the University of London, and his careful explanation of small detail shows his appreciation of the needs of students; but he has behind him an industrial career much longer and more distinguished than most professors or lecturers. This greatly enhances the value of his work, for he speaks with authority on the relative numerical importance of the terms in his equations, and is thereby enabled, by eliminating small effects, to present a clearer picture of the chief phenomena. His use of the 'per-unit' system is perhaps open to criticism in a students' book; it is easy to see its value to the designer, but it takes the student too far from fundamentals, destroys the dimensional consistency of the equations, and involves the author in quite a number of interpolated explanations of the values of coefficients. It is also possible to regret the somewhat disproportionate space accorded to the synchronous machine as compared with other types of a.c. motor. Nevertheless, the book is the best yet written in English to develop machine performance from general principles in a manner calculated to appeal to engineers and engineering students.

T. WILSON
D. A. JONES
G. W. CARTER

Science and Existence

Two Ways of Knowing. By Alan Richardson. (Technics and Purpose, No. 1.) Pp. 30.

Scientific and Technological Education

By Edwin Barker. (Technics and Purpose, No. 2.) Pp. 37.

Technical Education

By H. A. Warren. (Technics and Purpose, No. 3.) Pp. 37.

Purpose in Teacher Training

By Monica Wingate. (Technics and Purpose, No. 4.) Pp. 38.

(London: S.C.M. Press, Ltd., 1957.) Each 2*s*. 6*d*. net.

THE British Council of Churches is sponsoring a series of booklets to examine the issues arising out of the growth of science in society and the rapid expansion of technological education, particularly in relation to the purposes and values involved in the light of the Christian faith. For several years a working party convened by the Education Department of the British Council of Churches, under the chairmanship of Sir John Wolfenden, vice-chancellor of the University of Reading, has been meeting to consider some of the issues involved; it has now asked certain individuals to take responsibility for bringing together some of the views expressed in discussion. In the first booklet Prof. Alan Richardson, of the University of Nottingham, distinguishes with great clarity between 'scientific' and 'existential' knowledge. The narrowness of scientific and technological education is examined by Edwin Barker, assistant general secretary of the National Council of the Young Men's Christian Association, in the second booklet, while in the third, H. A. Warren, principal of the South-East London Technical College, describes the system and scope of technical education in England and Wales and makes an appraisal of some of its social and cultural effects. In the fourth booklet, Monica Wingate, principal of Balls Park Training College, searches out the purpose in teacher-training with irresistible persuasiveness; her readers might have been even more sympathetic if she had first made sure that they were aware of the ways in which teachers are trained in Britain's training colleges. Before other booklets in this series are produced, more thought might be given to the purpose behind each and to make this clear in a preface; the sequence in which they are issued should also be examined. T. H. HAWKINS

Malayan Orchid Hybrids

By M. R. Henderson and G. H. Addison. Pp. iv+191. (Singapore: Government Printer, 1956.) 21 Malayan dollars.

ALTHOUGH there is such a wealth of orchid hybrids available for cultivation in temperate countries, many of these are not happy when grown under tropical conditions. Successful efforts have therefore been made in recent years to develop hybrids within other orchid genera which are better suited to the tropics. These include the genus *Dendrobium* (particularly the section *Ceratobium*), the climbing orchids of the Vanda-Arachnis group, the strap-leaved *Vanda* species and *Spathoglottis*. The chief two centres in which this hybridization has been carried out are Malaya and the Hawaiian Islands. The present book aims at providing photographic illustrations of the more important and beautiful hybrids which have been raised, or are commonly cultivated, in Singapore.

For most varieties dealt with there are photographs of a single flower and of a complete inflorescence. These photographs reach a high standard and are excellently reproduced on art paper. Accompanying them are brief texts which include information about parentage and place of origin as well as short descriptions and notes on cultivation. Well-illustrated and concise accounts of the cultivation in Singapore of the main groups of hybrids precede each section of the book.

Orchid growers in Malaya and in the neighbouring countries will welcome this book and it will be of value to growers in other tropical regions. It will also serve to stabilize the nomenclature of these hybrids, to which additions are continually being made. Altogether the authors are to be congratulated on the production of a useful as well as very attractive book.

A Glossary of Mycology

By Walter H. Snell and Esther A. Dick. Pp. xxxi+171 (15 plates). (Cambridge, Mass.: Harvard University Press; London: Oxford University Press, 1957.) 40*s*. net.

MYCOLOGY, like all other sciences, has developed its own shorthand terms to describe the diversity of form, substance and function of fungal organisms. The present volume describes nearly seven thousand such terms, and, apart from the names of colours and certain words used in general botany, most have no meaning outside this science. This number of words is almost enough, in a simpler context, to found a language; have we too many of them? The majority save quite lengthy explanations, and so are justified as shorthand. A considerable number apply only in special parts of mycology, but this serves to indicate fungal complexity rather than redundant verbiage. A few terms, now obsolete but used in earlier literature, have historical value. A minute minority are colloquial. Colour names link those in the authoritative but relatively inaccessible "Repertoire de Couleurs" with other and more available specifications. Fifteen very clear line blocks by Henry A. C. Jackson elucidate many terms. The authors have, in all, performed a most necessary service, and have done it in a most scholarly manner.

J. GRAINGER

The Life of the Shrew

By Dr. Peter Crowcroft. Pp. vi+166. (London: Max Reinhardt, Ltd., 1957.) 15*s*. net.

THIS is an excellent account of the biology of the species of British shrews, with perhaps more emphasis on the common and pigmy shrews. Their general behaviour, fighting propensities, reproduction, activity rhythm and feeding, their longevity (or should it be brevity?) and their position both as predators and prey are all dealt with. Much is the result of the author's own observations and experiments; but all the previously known facts have been incorporated in a very readable book. The shrew's reputation in folk-lore and in naturalists' accounts, even as late as 1911, is shown to be largely erroneous. The gaps in our knowledge and the methods by which they may be filled are dealt with in a stimulating and attractive manner. There are a good bibliography and index, while the tailpieces by Erik Thorn portray the characteristic postures and behaviour of shrews most admirably. H. R. HEWER

ALPHA-RAY ACTIVITIES OF HUMANS AND THEIR ENVIRONMENT

By R. C. TURNER, J. M. RADLEY and Prof. W. V. MAYNEORD
Physics Department, Institute of Cancer Research: Royal Cancer Hospital, London, S.W.3

THE radioactivity of the human body and its environment has become a matter of increasing importance and concern particularly in view of the hazards now known to be associated with the ingestion or inhalation of small quantities of radioactivity. It is well known that the human body normally contains small quantities of naturally occurring radioactive elements particularly concentrated in bone[1-3]; but to what extent these materials are derived from foodstuffs, drinking waters, or other ingested substances, is largely unknown for a particular individual. The knowledge of the natural radioactivity of our environment is so scanty that it seemed possible that a number of natural sources of irradiation of human beings exist of which we are, at the moment, ignorant. Although largely unknown, these levels of radioactivity have been assumed to be of rather academic importance, yet they form the only sure ground upon which we may argue as to the dangers of different levels. For example, the natural background of γ-rays gives us a standard by which we may judge the possible effects of increased radiation on the genetic material of the race, while the natural radiation to which bone for many generations has been exposed gives us a basis on which to judge any possible increase in the production of bone tumours or leukæmia. Since many of the naturally occurring radioactive nuclides emit α-particles it seemed worth while to look again for such α-activities both within the human body and its environment. This article outlines the results of investigations over a fairly wide range on many hundreds of samples.

Experimental Technique

We have devised a simple method which enables us to measure α-activities without elaborate chemical preparation[4].

The material, the activity of which is to be measured, is often human or animal tissue and is normally prepared by ashing at 600–700° C., and grinding the residue to a powder. A shallow translucent tray is formed by stretching Scotch tape over one surface of a 'Perspex' ring about 11 cm. internal diameter and about 3 mm. thick. The inside adhesive surface of the tray so formed is covered with a thin layer of finely powdered nickel-killed zinc cadmium sulphide which serves as the α-detecting phosphor. The tray is filled with the powdered sample and is sealed by further layers of Scotch tape stretched across its upper surface. Such a tray holds 2–3 gm. of unit density material of which only about 150 mgm. contribute to the counting, the precise amount depending, of course, upon the range and therefore the nuclide from which the α-particles come. This sealed tray ensures freedom from contamination. Samples may be stored indefinitely, and repeated measurements made at appropriate intervals, the rate of rise of activity to equilibrium after sealing yielding important data in identification of nuclides present. The sealed sample is presented to the photo-cathode of a multiplier and the α-particles counted by suitable techniques. The lowest backgrounds observed by us were obtained using samples of aged distilled water, and are approximately 3 counts per hour in counting periods extending over a week. The sensitivity of the method may be gauged from the fact that 1 count per hour above the background corresponds to about 4×10^{-14} c./gm. of sample and thus enables the normal α-activity in bone ash (that is to say, about 2×10^{-13} c./gm.) to be determined to an accuracy of some 15 per cent in a counting time of 24 hr. Details of the technique will be published elsewhere[4]; but it is obvious that great care has to be taken to avoid contamination. The method has been modified to measure the activities of solid, liquid or gaseous samples. A study of the elementary geometry of counting shows that the number of particles (N) emitted per unit volume per unit time and the range (R) of the particles in the material are the determining constants, and that the product $NR/4$ gives the number of particles per cm.^{2} which are observed in a given time. We have discussed in detail[4] the effects of density and of atomic number on the range of the particles and calculated mean ranges in media of varying atomic numbers and densities.

Early in the experiments it was observed that materials containing thorium and its daughters, at low counting rates of the order of 10–100 counts per hour, yielded a number of closely spaced pairs of α-particles. These arise from the two consecutive α-transitions thoron (220) to thorium A (216), and thence to thorium B (212). Since the half-life of thorium A (216) is only 0·14 sec., and since no analogous phenomenon occurs in the radium series, the frequency of occurrence of these fast pairs gives a direct measure of the rate of disintegration of thoron atoms. The results must, of course, be corrected for the chance occurrence of spurious pairs. By a development of this argument it is possible to estimate what fraction of the observed total α-count arises from the uranium and from the thorium series respectively. This phenomenon, well known in the history of radioactivity[5], has enabled us to demonstrate and measure the fraction of the activity of normal human bone which arises from the thorium series.

Abnormal Radioactive Contents in Humans

Some of our first experiments were carried out on samples of the humerus from a radium worker who ingested active material around the period 1910. The observed counting rates were very high, 17,500 counts per hour in our normal sample, and the resulting activity of $4{\cdot}70 \times 10^{-11}$ gm. radium-226 per gm. wet bone was in good agreement with estimations

of radium which had previously been made by pressure ionization chamber techniques, autoradiography, physical-chemical assays and excreted radon[6]. We have also measured a number of samples of humerus from a worker who ingested both radium and mesothorium 8–10 years earlier. Again, the estimated ratio of radiothorium to radium was in good agreement with previous measurements, as were the absolute values of $5{\cdot}3 \times 10^{-12}$ c. and $5{\cdot}9 \times 10^{-12}$ c./gm. wet bone respectively (Spiers, F. W., private communication). It was established that other bones of the same subject (ribs and sternum) gave values for the radiothorium ranging from $6{\cdot}0 \times 10^{-12}$ c. to $5{\cdot}3 \times 10^{-12}$ c. per gram of wet bone, but the ratio of radiothorium to radium (0·89) was surprisingly constant throughout the various parts of the skeleton.

As a further example of the flexibility of the technique we may mention that we have measured the breath and the blood of a patient who was given 'Thorotrast' for diagnostic reasons some twenty years ago. The estimated breath activity was $1{\cdot}5 \times 10^{-9}$ c. thoron per litre and the blood content of thorium $B = 3{\cdot}5 \times 10^{-12}$ c./cm.^{3}.

Normal Radioactivity in Humans

The cases we have quoted are of patients having very large radioactive burdens perhaps ten thousand times greater than those of the general population, and we now turn to studies designed to find the mean level of α-activity of normal populations. We were particularly anxious to obtain data concerning young children since little information exists as to the concentrations of radioactive material present at birth. We have, therefore, made measurements on as much stillborn material as could be obtained.

Observations have been made on the α-activities of bones of persons native to Cornwall, London and Cumberland, respectively. 31 Cornish (humerus), 17 Cumberland (femur) and 12 London (humerus) bone specimens have been examined. In the case of the 11 stillborn children, all of whom were full-term, low calcification and small size of the bones make it necessary to ash the whole of one of the long bones, in this case the femur.

The results of the human bone measurements are shown in Fig. 1. It will be observed that there is great variation in absolute level, and that on the whole Cornish bones have higher values than those observed from the London area or from Cumberland. The mean content of the Cornish material was $5{\cdot}0 \times 10^{-12}$ c. of α-activity per gm. bone ash compared with $1{\cdot}7 \times 10^{-12}$ c. for the London area. This difference appears to be statistically significant; but the question remains as to whether the subjects form representative samples of the inhabitants of the two regions. The values could be influenced by geographical factors, and also by the fact that a number of the specimens drawn from the London area were cases of advanced malignant disease known to have low calcium content. It will be shown later that differences in feeding habits could also be very significant. One of the most interesting findings is the unexpectedly high value for the concentration of α-activity of stillborn children[7], the mean value in our experiments being $4{\cdot}5 \times 10^{-12}$ c. per gm. bone ash. It appears that in adult bone approximately 40 per cent of the α-activity is due to the radiothorium series. For the 11 stillborn children the mean fraction is the same, a result which is particularly interesting.

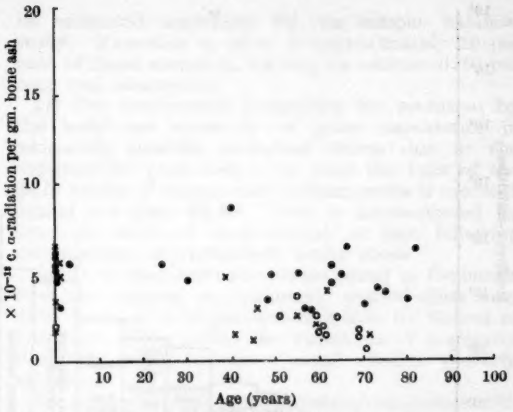


Fig. 1. Human bone ash, total α-activity. ●, Cornwall; ×, London; ○, Cumberland

The other striking feature of Fig. 1 is that there is no obvious correlation of α-activity with age. In view of the great interest in the radioactivity of children we have measured four samples of liquid milk and found a mean value of $3{\cdot}1 \times 10^{-12}$ c. per gm. ash. This implies that the total α-content and the mean radium content of a pint of milk from the London area are, respectively, 1·3 μμc. per pint and 0·22 μμc. of radium per pint. In a few months the total intake of the human infant, if completely incorporated into the body, would be sufficient to provide most of the radium found stored in the adult. Later experiments suggest, however, that a large fraction may be excreted.

We have also measured the total α-content of two samples of human mothers' milk ash, and obtained values of $7{\cdot}9 \times 10^{-13}$ and $3{\cdot}1 \times 10^{-13}$ c. of total α-activity per gm. ash. The activity appears to be divided about evenly between radium and radiothorium.

Radioactivity in Animals

It seemed natural in view of these activities in milk to study the radioactivity of cows. Measurements of samples of long bones of 18 cows yielded values of total α-activity ranging from $2{\cdot}0 \times 10^{-12}$ c./gm. ash to $6{\cdot}0 \times 10^{-12}$ c./gm. ash with a mean of $3{\cdot}0 \times 10^{-12}$ c./gm. ash. It will be seen that the mean total α-activity per gm. of ash for cows is about ten times that of the humans. One very high activity in a 'marrow bone' ($1{\cdot}0 \times 10^{-11}$ c. per gm. ash) is interesting, as also is the observation that the radioactivity in the bone of cows consists of about equal parts of radium and thorium series. By ashing the bone marrow of cows separately we found that the activity of the bone marrow is the same as the activity of the bone (c. per gm. ash) but relatively rich (2 : 1) in radiothorium compared with radium. In further experiments we have measured the activities of cow spleens and found a mean value of $1{\cdot}8 \times 10^{-12}$ c./gm. spleen ash, again, with about equal parts of the thorium and radium series. The ratio of α-activity in milk to the activity appearing in bone is very different in humans and cows, the ratio being at least ten times higher for humans. These observations suggest interesting comparative physiology of different species.

It may be of interest to mention that we have also made preliminary observations on some other

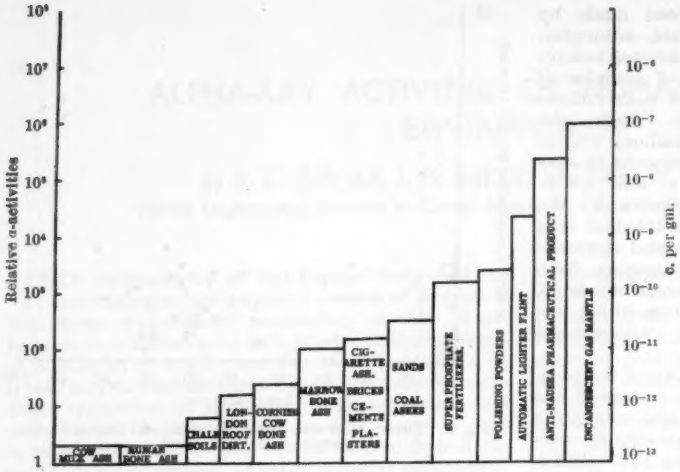


Fig. 2

species, as, for example, bones of five sheep which show values between 2 and 6×10^{-12} c. per gm. ash. Observations on the bones of pig reveal very low activity, while the bones of a horse, 18 years of age, are also quite low at $3{\cdot}8 \times 10^{-13}$ c. per gm. ash. It is evident there are differences between the natural radioactive content of bones of various species, but we are at the moment ignorant as to whether or not these are real species differences or determined by feeding habits.

Soils

We have also made observations on forty types of soils and fertilizers. They were not necessarily representative, but grab samples taken in various ways. We have found that chalk is low in activity (7 to 8×10^{-13} c. of α-activity per gm.), a fact of great importance to us in the design of new low background laboratories. The next lowest soils are the limestones, as is indeed well known[8]. One of the most active materials we have encountered is fossil superphosphate (1 to 2×10^{-11} c. α-activity per gm.), but again these activities were well known and may be so high that in the United States, for example, the extraction of uranium from superphosphate residues is worthwhile commercially[9]. The high values for superphosphates may have a bearing upon the observed activities of cattle bones.

Various Environmental Materials

In another series of experiments α-activities have been found in a number of substances in which thorium and rare earths are closely associated. It is not uncommon for thorium to pass through the chemical separation processes in company with materials like cerium, thus giving rise to the activities we have observed. Among the materials examined, conspicuous ones are lighter flints (3×10^{-9} c. α-activity per gm.), cleaning materials of various kinds involving cerium (4×10^{-10} c. α-activity per gm.) and in one instance a pharmaceutical product which evidently contains a large percentage of thorium (Fig. 2).

Table 1

Foodstuff	Maximum α-activity observed per 100 gm. $\times 10^{-12}$ c.
Cereals (breakfast)	60
Liver and kidney	15
Flours	14
Chocolates	8
Biscuits	2
Milks (evaporated)	1-2
Fish	1-2
Vegetables	0·7
Meat	0·5
Fruit (tinned)	0·5

Human Metabolic Experiments

In view of the extremely wide range of naturally occurring activities in the human environment it seemed of great importance to discover the immediate sources of radioactivity of the normal human. We have, therefore, carried out a 'balance' experiment on a normal young human subject with the view of investigating the magnitude of the intake and excretion of naturally occurring radioactive material.

Samples of every food eaten by a boy (4 years old) over a period of a month were assayed for α-activity,

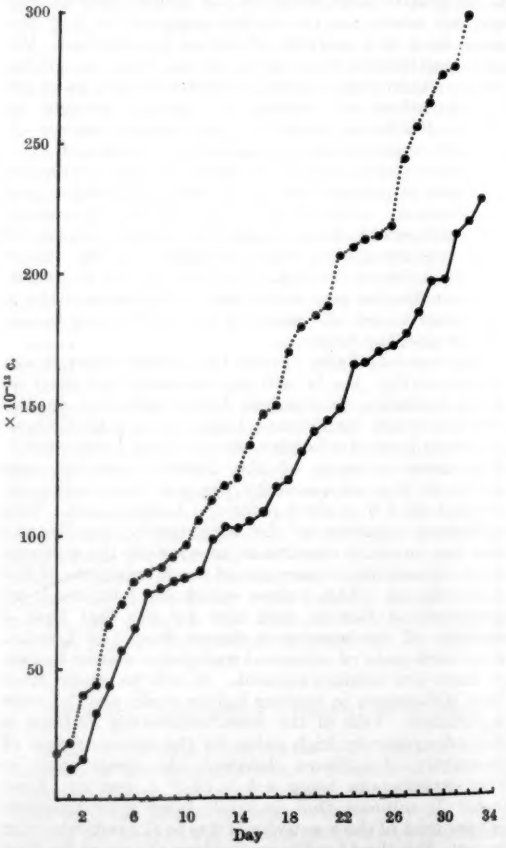


Fig. 3. ●···●, Intake, total α-activity, $299{\cdot}2 \times 10^{-12}$ c.; ●—●, faecal excretion, total α-activity, $228{\cdot}8 \times 10^{-12}$ c.

as was each daily fæcal sample excreted during that time. It was immediately found that not only were the fæcal samples easily measurable but also examination of a number of foodstuffs revealed variations in α-activity ranging over factors of 4–5 hundred times (Table 1 contains a small selection of those found relatively active). The intake over a period of a month was $3{\cdot}0 \times 10^{-10}$ c. of α-activity with the fæcal excretion 20 per cent lower. Fig. 3 shows the integrated daily intakes and fæcal excretions.

The main conclusions from this pilot experiment are:

(*a*) Food is, in this instance, the important factor determining the daily intake of naturally occurring radioactive material, and its contribution completely overshadows the intake from milk and water, hitherto often supposed the main sources.

(*b*) The levels of α-activity in foodstuffs are extremely variable but, in general, high values are obtained for cereals and flours, particularly those in which whole wheat is utilized.

(*c*) The intake per day from food, even for a child of 4 years of age, may approximate the whole body burden. For an adult, the intake can exceed 10^{-10} c. of α-activity per day.

(*d*) The ratios of radiothorium to radium in the intake and in the fæcal excretion are substantially the same (0·75). This ratio is higher than that found in the human bone studies (0·6) possibly because of the relatively higher values of radiothorium in such organs as the liver and spleen.

(*e*) The daily retention of radiothorium and radium is evidently a small fraction of the intake but cannot be estimated accurately by our simple 'balance' study. Excretion in urine is approximately 10 per cent of fæcal excretion, leaving an estimated 10 per cent real absorption.

(*f*) The mechanisms controlling the retention by the body are evidently of great importance in estimating possible biological effects due to the radioactivity presented to it, since the bulk of the daily intake of radium and thorium series is excreted during the next 48 hr. This is demonstrated by the high values of fæcal activity on days following the ingestion of particularly active foods.

(*g*) It is clear from the values found in foodstuffs that two persons on apparently normal diets may differ in total daily radioactive intake by factors of hundreds. This makes the variations of α-activity observed in the human skeletal studies scarcely surprising.

We are continuing these physiological studies with the view of a better understanding of the metabolism and resulting dosimetry due to natural and artificial radioactive substances.

[1] *Brit. J. Radiol.*, Supp. No. 7 (1957).
[2] "The Hazards to Man of Nuclear and Allied Radiations", Medical Research Council (H.M.S.O., London, 1956. Cmd. 9780).
[3] *Brit. J. Radiol.*, Supp. No. 6 (1955).
[4] Turner, R. C., Mayneord, W. V., and Radley, J. M., *Brit. J. Radiol.* (in the press).
[5] Rutherford, E., "Radioactive Substances and their Radiations", 395 (Cambridge University Press, 1913).
[6] *Brit. J. Radiol.*, Supp. No. 7, 81, 92 (1957).
[7] Hursh, J. B., and Gates, A. A., *Nucleonics*, **7**, 46 (1950).
[8] Meyer, S., and Schweidler, E., "Radioactivität", 546 (Teubner, Leipzig, 1927). Ponsford, D. R. A., Bull. Geol. Surv. Great Britain, No. 10, 24.
[9] Bowie, S. H. U., "Atomic Energy", a *Financial Times* Survey, 15 (April 1956).

FORMATION OF TWO- AND THREE-STRANDED HELICAL MOLECULES BY POLYINOSINIC ACID AND POLYADENYLIC ACID

By Dr. ALEXANDER RICH

Section on Physical Chemistry, National Institute of Mental Health, Bethesda, Maryland

A GREAT deal of interest has been aroused by problems associated with the structure and function of ribonucleic acid. This substance is found widely distributed in various parts of the cell. Although some of it is in the nucleus, where it is near the deoxyribonucleic acid, most of the ribonucleic acid is in the cytoplasm, where it is either closely associated with protein to form the particles in the microsomal fraction or is free in the supernatant. Because it constitutes almost half the microsomal particles in which protein synthesis is carried out[1], it is widely believed that it takes a fundamental role in this process, such as helping to determine the sequence of amino-acids in the newly synthesized protein. It has also been demonstrated that ribonucleic acid can function as a carrier of genetic information. If ribonucleic acid is isolated from the tobacco mosaic virus, it is capable of infecting the tobacco leaf and thereby producing a large number of new virus particles which carry the genetic markers of the original virus[2].

Studies of the molecular structure of ribonucleic acid have been carried out in the hope of learning something about the molecular mechanisms involved in these processes. However, until recently, it has been extremely difficult to obtain reliable structural information. Ribonucleic acid is somewhat unstable chemically, and most isolation procedures result in a degraded product so that we have only meagre information about the configuration of this substance[3].

However, a number of recent developments have made more promising the prospect of solving this problem. Grunberg-Manago and Ochoa[4] have discovered an enzyme which is capable of converting nucleotide diphosphates into polyribonucleotides. The resultant polymers resemble naturally occurring ribonucleic acid in that they have the same covalent ribose-phosphate backbone, and they have been shown to undergo a similar enzymatic hydrolysis[5]. X-ray diffraction studies of polymers containing all four of the purine and pyrimidine bases present in ribonucleic acid produce an X-ray diffraction pattern

which is virtually identical with that produced by the naturally occurring substance[6]. Hence, these materials are ideal analogues for studying molecular configuration problems associated with ribonucleic acid. Since it is possible to control the type of purine or pyrimidine base in the polymer, we can learn how the base composition influences the configuration of the ribose-phosphate backbone. Of special interest, of course, are the hydrogen-bonding potentialities of the various bases, since the specificity of these interactions takes such a crucial role in maintaining the structure of deoxyribonucleic acid[7].

Polyadenylic Acid and Polyuridylic Acid

Among the first combinations of synthetic polynucleotides to be investigated structurally was polyadenylic acid plus polyuridylic acid. When these two elongated polymeric species meet in solution, they wrap around each other to form a two-stranded helical molecule, one strand of which is polyadenylic acid and the other polyuridylic acid[8]. The resultant 1 : 1 complex can be identified by its absorption spectrum in the ultra-violet as well as through its X-ray diffraction pattern. This diffraction pattern has many features in common with that produced by deoxyribonucleic acid, and it is believed that the adenine and uracil residues are joined together by the same hydrogen-bonding system which is found in deoxyribonucleic acid. This work further suggested that the ribonucleic acid backbone is capable of assuming the deoxyribonucleic acid configuration, that is, a helical form with the phosphate groups outside and a translation of 3·4 A., and a screw rotation of 36° between the hydrogen-bonded base pairs. The presence of the additional hydroxyl group on C'_2 of ribose modifies the configuration only slightly in this case.

An additional interaction was discovered between the two-stranded polyadenylic acid + polyuridylic acid molecule and a third strand of polyuridylic acid[9]. It has been shown that, in the presence of 0·012 *M* magnesium chloride, these interact in the ratio of one polyadenylic acid to two polyuridylic acid molecules, as measured by the ratio of reacting nucleotides. The interaction is accompanied by a change in the ultra-violet spectrum and sedimentation constant of the resultant material. It is believed that the third strand of polyuridylic acid has wrapped itself about the two-stranded polyadenylic acid + polyuridylic acid molecule, filling one of the helical grooves. Recently, a diffraction pattern has been obtained from material which was prepared under conditions in which the three-stranded complex forms. This pattern differs from that obtained with the two-stranded complex (unpublished work).

These results led to a search for other two- and three-stranded configurations among the synthetic polynucleotides. This communication reports the discovery of a new two-stranded helical configuration involving polyadenylic acid and polyinosinic acid, as well as a three-stranded complex involving an additional polyinosinic acid.

Polyinosinic Acid and Polyadenylic Acid

At the beginning of this investigation, I was informed by Prof. Paul Doty, of Harvard University, that he had observed a "helix-coil transition" in the system polyadenylic acid and polyinosinic acid (personal communication).

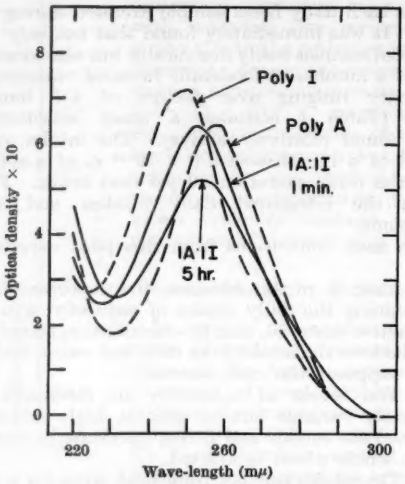


Fig. 1. Broken lines show the absorption spectra of polyadenylic acid (poly A) and polyinosinic acid (poly I) before mixing. The solutions are both in 0·05 *M* sodium chloride, 0·001 *M* sodium cacodylate at *p*H 6·8, $T = 23°$ C. The solid lines show the absorption spectrum of a 1 : 1 mixture (1 A : 1 I) 1 min. after mixing, and at 5 hr. As the complex forms, there is a drop in the optical density and a shift of the maximum and minimum

The polynucleotides used in my investigation were polymerized from nucleotide diphosphates and the enzyme polynucleotide phosphorylase from *Azotobacter vinelandii*. The highly purified enzyme (specific activity 113 units) was kindly supplied to me by Prof. S. Ochoa. Polymers were prepared during a two-hour incubation period and harvested by precipitation with cold ethanol followed by dialysis at 5° C. against 5×10^{-3} *M* sodium chloride and subsequent lyophilization. The sodium salt of the polynucleotides was used for all experiments.

Extinction coefficients were measured by assaying the ribose content of the solutions[10]. In 0·1 *M* sodium chloride, 0·01 *M* sodium cacodylate at *p*H 6·8, the molar extinction coefficient per nucleotide for polyadenylic acid is 9,180 at 259 mμ, and for polyinosinic acid it is 10,300 at 250 mμ.

The interaction between these two molecular species is best seen spectrophotometrically. In Fig. 1, the ultra-violet absorption spectra are plotted for equal concentrations of the individual polymers (dashed lines). These solutions are both in 0·05 *M* sodium chloride, 0·001 *M* sodium cacodylate at *p*H 6·8. When they are mixed together in a 1 : 1 ratio, spectra are observed as shown by the solid lines. At 1 min. after mixing, the resultant absorption spectrum curve lies half-way between the curves for the individual materials. However, the resultant spectrum changes with time. Equilibrium is obtained by 5 hr., at which time the final curve has dropped considerably as shown in Fig. 1. At equilibrium, the spectrum has shifted somewhat and has a new maximum at 254 mμ and a minimum at 231 mμ. The final optical density at the maximum has decreased by 18 per cent when compared to the starting point, and half this drop has occurred in approximately 20 min. under these conditions. Such a lowering of the optical density is called the 'hypochromic effect' and usually refers to the decrease in optical density which is attendant on the polymerization of nucleotides to form nucleic acids. In the synthetic polynucleotides, changes in the hypochromic effect

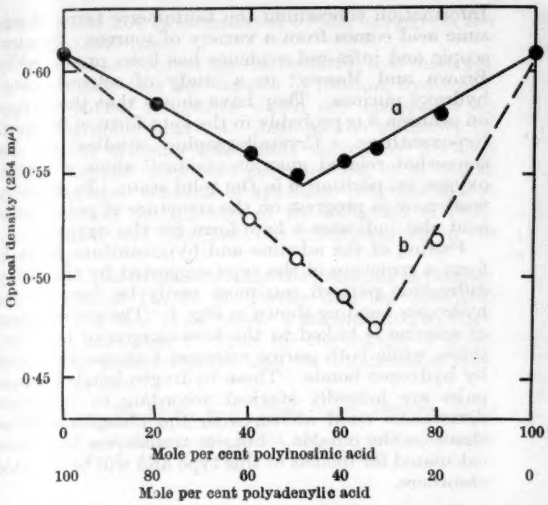


Fig. 2. Optical density at 254 mμ of mixtures of polyinosinic acid and polyadenylic acid. The conditions are the same as in Fig. 1. All points refer to the same total concentration of nucleotide phosphate. The solid curve (*a*) is obtained at 31 min. and the broken curve (*b*) at 5 hr. The minimum shifts from 50 per cent polyinosinic acid in (*a*) to 67 per cent in (*b*)

have been used as a convenient index for following the course of a reaction. Thus, for example, the reaction between polyadenylic and polyuridylic acids results in a similar lowering of the optical density in the ultra-violet[8,11,12].

The stoichiometry of the reaction can be determined by measuring the drop in optical density at a given wave-length as a function of composition. In Fig. 2, the optical density at 254 mμ is plotted as a function of mole ratio of polyadenylic acid to polyinosinic acid. The upper curve, *a*, is obtained 31 min. after mixing two standard solutions, both of which are in 0·05 *M* sodium chloride, 0·001 *M* sodium cacodylate, *p*H 6·8. In all these mixtures, the total concentration of nucleotide phosphate was kept constant, but the relative amounts of the two species vary. At 31 min. a minimum in the optical density is seen near 50 per cent, that is, a 1 : 1 mixture of polyinosinic and polyadenylic acids.

However, if these same mixtures are allowed to stand until equilibrium is reached, the curve changes further (Fig. 2, *b*). At equilibrium, the minimum has shifted to 67 per cent polyinosinic acid, suggesting the formation of a 2 : 1 complex involving two polyinosinic acid molecules and one polyadenylic acid molecule.

The formation of these two complexes is controlled by the ionic strength of the medium. As the salt concentration is decreased, the rate of the reaction decreases. Thus, in 0·01 *M* sodium cacodylate without added sodium chloride, only the 1 : 1 complex forms (Fig. 2, *a*) after 18 hr. On the other hand, in 0·1 *M* sodium chloride, the minimum at 50 per cent is seen clearly only until 40 sec. after mixing. Following that, the optical density – composition curve begins to shift, and the sharp minimum at 66 per cent polyinosinic acid is seen clearly after 4 min. At very high salt concentrations, for example, 1 *M* sodium chloride, no reactions occur. This is probably due to an alteration in the polyinosinic acid molecule and will be discussed in greater detail in another paper dealing with the structure of polyinosinic acid.

Further evidence for the formation of these complexes can be obtained from an ultracentrifuge investigation. For these studies, a 'Spinco' model *E* ultracentrifuge was used in a form modified for ultra-violet absorption measurements. In this way, concentrations were used near 6×10^{-5} *M* in nucleotide phosphate and sedimentation constants were independent of concentration. Polyadenylic acid in 0·05 *M* sodium chloride and 0·001 *M* sodium cacodylate at *p*H 6·8 had a mean sedimentation coefficient (S_{20}) of 7·2, whereas the polyinosinic acid had a value of 8·6 under the same conditions. A 1 : 1 mixture of these had an average $S_{20} = 10{\cdot}4$ at equilibrium. However, an equilibrium mixture of 2 polyinosinic acid to 1 polyadenylic acid had an average $S_{20} = 14{\cdot}7$.

The experiments cited above demonstrate the formation of complexes between polyadenylic and polyinosinic acid. In order to obtain information about the detailed molecular configuration of these complexes, an X-ray diffraction investigation was carried out.

X-Ray Diffraction Studies

A 1 : 1 mixture of sodium salts of polyadenylic acid and polyinosinic acid was prepared by adding together weighed amounts of the dry material. The mixture was dissolved in distilled water without any added salt. Under these conditions, the solution had only the residual sodium chloride from the final dialysis in the preparation of the polymers. At this low salt concentration, only the 1 : 1 complex forms, as described above. After reacting at room temperature for one hour, the solution was lyophilized. The resultant dried fibrous mixture was then moistened until it became sticky and from this were drawn long, tough fibres which upon drying became brittle and highly birefringent ($\Delta n = -0{\cdot}09$). X-ray diffraction photographs were taken using an intense beam of X-rays 100μ in diameter (Hilger 'Semi-Micro' X-ray Tube Stand).

Fig. 3 shows a diffraction photograph obtained at 66 per cent relative humidity in a helium-filled camera. The distribution of scattering intensity is characteristic of a helical structure, as shown by the fact that the innermost maxima on the first, second, and third layer lines lie approximately on straight lines radiating from the origin. In addition, there is a large area on the meridian which is empty. In some respects, the diffraction photograph is similar to that produced by the *B* form of deoxyribonucleic acid[13].

There are two fairly strong reflexions on the equator and several weaker ones. These appear at distances which indicate a hexagonal lattice with $a = 24{\cdot}4$ A. By measuring the layer line spacing, it can be shown that the pitch of the helix is 38·8 A. The specimen was tilted slightly in Fig. 3 to show the upper layer lines more clearly. Several layer lines appear near the meridian in the region of 3–4 A. From these it appears that there is a meridional reflexion near 3·4 A. Hence the fundamental screw repeat for the helix is a translation of 3·4 A. and a rotation of

$$\left(360° \times \frac{3{\cdot}4}{38{\cdot}8}\right) = 31{\cdot}5°$$

The reflexions in the 3·4-A. region are undoubtedly due to the stacking of the planar purine bases at right angles to the fibre axis. This interpretation is

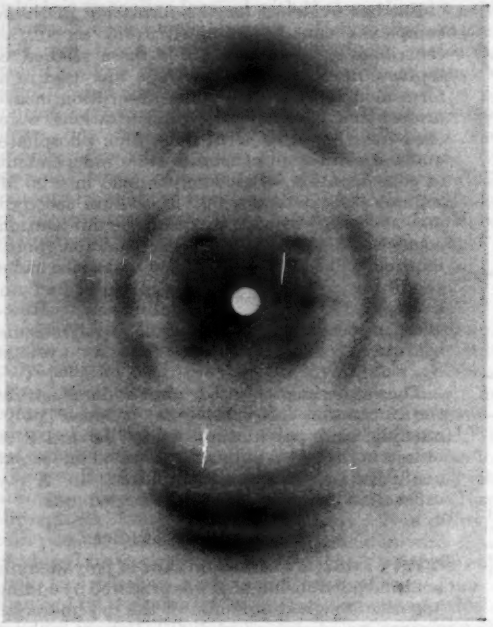


Fig. 3. X-ray diffraction photograph of a fibre of polyadenylic acid plus polyinosinic acid (1 : 1). The fibre is at a relative humidity of 66 per cent in a helium-filled camera, and is tilted slightly from the vertical

compatible with the observed high negative birefringence.

Cochran, Crick and Vand have analysed the diffraction patterns produced by helical molecules[14]. They have shown that the structure factor for a given layer line is the sum of a series of terms each of which contains a Bessel function $J_n(2\pi rR)$, where r is the radius of an atom, and R is the radius in reciprocal space. In the nucleic acids, the diffraction pattern is usually dominated by the contributions of the phosphate groups; hence it is possible to carry out a very approximate analysis by considering their contributions alone. In this approximate analysis the positions of the innermost maxima on layer lines 1, 2, and 3 can be equated to the first maxima of $J_n(2\pi rR)$, where n is 1, 2 or 3, respectively. Such an analysis yields a weighted 'average' scattering radius of 9·5 A. This strongly suggests that the phosphate groups are on the outside of the molecule, as in deoxyribonucleic acid.

Discussion

With the ribose-phosphate backbone helically arranged on the outside of the two-stranded complex, it is necessary to determine the arrangement of the two purine bases relative to each other in the centre of the molecule. The diffraction data clearly indicate that these planar groups are oriented at right angles to the fibre axis, and it is necessary to consider the manner in which they might be linked together by hydrogen bonds. Before this can be done, one must know the tautomeric forms which exist under the conditions in which the polynucleotides interact.

It has been generally assumed that the adenine residue in the nucleic acids is in the amino-form. A recent study of the infra-red spectrum of adenylic acid in solution has confirmed this assignment[15]. Information concerning the tautomeric form of inosinic acid comes from a variety of sources. Spectroscopic and infra-red evidence has been presented by Brown and Mason[16] in a study of several monohydroxy purines. They have shown that the oxygen on position 6 is probably in the keto-form in 9-methyl hypoxanthine. Crystallographic studies on the somewhat related guanine residue[17] show a ketonic oxygen on position 6 in the solid state. In addition, work now in progress on the structure of polyinosinic acid also indicates a keto-form for the oxygen.

Pairing of the adenine and hypoxanthine bases to form a structure of the type suggested by the X-ray diffraction pattern can most easily be done by the hydrogen-bonding shown in Fig. 4. The amino-group of adenine is linked to the keto-oxygen of hypoxanthine, while both purine nitrogen 1 atoms are linked by hydrogen bonds. These hydrogen-bonded purine pairs are helically stacked according to the screw dimensions cited above, with the phosphate ribose chain on the outside. Fourier transforms have been calculated for models of this type and will be reported elsewhere.

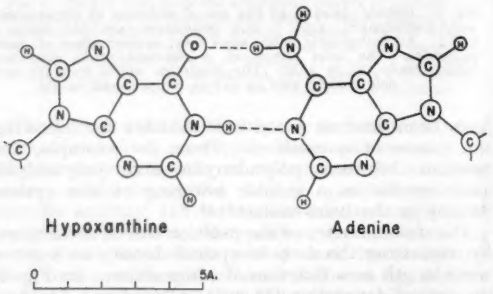


Fig. 4. Hydrogen bonding between hypoxanthine and adenine bases. One carbon atom from the ribose sugar is shown

At the present time, there is only spectroscopic and ultra-centrifugal evidence for the existence of the three-stranded complex. However, we know that the two-stranded molecule is helical, and by analogy with the system of polyadenylic acid plus polyuridylic acid, it is quite likely that the third strand lies in the helical groove of the two-stranded molecule. It is possible, for example, that the second hypoxanthine base is linked to the adenine residue by two hydrogen bonds (hypoxanthine keto-oxygen and nitrogen 1 bonded, respectively, to adenine amino-nitrogen and nitrogen 7 of the imidazole ring). A somewhat similar arrangement has been suggested for the second uracil chain, which is bonded to the two-stranded polyadenylic acid plus polyuridylic acid complex[9].

While inosinic acid is believed to be a precursor of adenylic acid as well as guanylic acid, it has not been found in ribonucleic acid itself. However, it is related structurally to guanylic acid in that it has the same keto-oxygen on carbon 6 of the purine ring, even though it lacks the amino-group on carbon 2. It is less closely related to adenylic acid, since substituting a keto-group for an amino-group radically alters the hydrogen bonding possibilities of the purine. This is, of course, readily seen by noting the great difference between the diffraction pattern of polyinosinic acid + polyadenylic acid relative to that of polyadenylic acid by itself. In the latter case, the structure is believed to be a two-stranded helix with each chain organized around an axial two-fold rotation axis[11]. The amino-group has a position in that structure

which could not be substituted by the oxygen on the hypoxanthine base.

The studies reported here have shown that mixtures of polynucleotides are capable of forming hydrogen-bonded helical structures which are susceptible to crystallographic analysis. Just as the structure of the fibrous proteins was finally solved by working on the simpler synthetic polypeptides, it is hoped that structural analysis of synthetic polyribonucleotides will aid the solution of the structure of ribonucleic acid and facilitate our understanding of its function.

I would like to acknowledge the generous assistance and advice of Dr. D. R. Davies and the technical assistance of Mrs. J. Johnson. I am indebted to Prof. S. Ochoa and Miss P. Ortiz for the supply of polynucleotide phosphorylase, and for information concerning its use.

[1] Littlefield, J. W., Keller, E. B., Gross, J., and Zamecnik, P. C., *J. Biol. Chem.*, **217**, 111 (1955).
[2] Gierer, A., and Schramm, G., *Nature*, **177**, 702 (1956). Fraenkel-Conrat, H., *J. Amer. Chem. Soc.*, **78**, 882 (1956).
[3] Rich, A., and Watson, J. D., *Nature*, **173**, 995 (1954); *Proc. U.S. Nat. Acad. Sci.*, **40**, 759 (1954).
[4] Grunberg-Manago, M., and Ochoa, S., *J. Amer. Chem. Soc.*, **77**, 3165 (1955).
[5] Heppel, L., Ortiz, P., and Ochoa, S., *J. Biol. Chem.* (Dec. 1957).
[6] Rich, A., *Ann. N.Y. Acad. Sci.* (in the press).
[7] Watson, J. D., and Crick, F. H. C., *Nature*, **171**, 737 (1953).
[8] Rich, A., and Davies, D. R., *J. Amer. Chem. Soc.*, **78**, 3548 (1956).
[9] Felsenfeld, G., Davies, D. R., and Rich, A., *J. Amer. Chem. Soc.*, **79**, 2023 (1957).
[10] Mejbaum, W., *Z. physiol. Chem.*, **258**, 117 (1939).
[11] Warner, R. C., *Fed. Proc.*, **15**, 379 (1956).
[12] Felsenfeld, G., and Rich, A., *Biochim. Biophys. Acta* (in the press).
[13] Wilkins, M. H. F., Stokes, A. R., and Wilson, H. R., *Nature*, **171**, 738 (1953). Franklin, R. E., and Gosling, R. G., *Nature*, **171**, 739 (1953).
[14] Cochran, W., Crick, F. H. C., and Vand, V., *Acta Cryst.*, **5**, 581 (1952).
[15] Miles, H. T., *Biochim. Biophys. Acta* (in the press).
[16] Brown, D. J., and Mason, S. F., *J. Chem. Soc.*, 682 (1957).
[17] Broomhead, J. M., *Acta Cryst.*, **4**, 92 (1951).
[18] This work is to be published by Crick, F. H. C., Watson, J. D., Davies, D. R., and Rich, A. Preliminary descriptions of the structure have appeared in "The Chemical Basis of Heredity" (Johns Hopkins Press, Baltimore, 1957), p. 552, by Watson, J. D., and in ref. 6.

THE BAND THEORY OF METALS

A SHORT and valuable conference was held in the Imperial College of Science and Technology, London, on December 19 and 20 on "The Band Theory of Metals and the Structure of the Fermi Surface". It was attended by about 240 members of the Physical Society and visitors, including Prof. Vonsovski from the U.S.S.R. The chief value of the conference must lie in the way in which it has drawn together many aspects of the subject. In the years 1928–35, almost the whole theory of metals was fairly satisfactorily sketched in outline, and we were often reminded of this during the conference. Since then, much work has gone into filling in the many gaps that remained. The different threads have now been drawn together again into a more comprehensive theory of metals and into a united research front. There were several review papers of various experimental techniques and theoretical developments, emphasizing what relevance the results obtained from them had for the theory of metals as a whole. There was also a good mixture of theoretical and experimental papers, and much emphasis on turning all this to good account by trying to understand, in detail, the properties of at least a few individual metals. As a further elaboration of this theme, the following thoughts remained in the mind of one participant after the conference. In some places the remarks go beyond what was actually said in the formal sessions.

Prof. N. F. Mott opened the conference with a paper on "Band Theory and Ferromagnetism", which brought out the distinct types of wave function that we use in different circumstances to describe electrons in metals. In the earliest development of the subject, the wave functions of the individual electrons were taken as waves $\psi_{\mathbf{k}}$ (with wave vector $\mathbf{k}$) extending throughout the crystal, and the wave function of the whole metal was written as a product or as a single determinant :

$$\Psi_{\text{Bloch}} = (N!)^{-1/2} \mid \text{determinant of the } \psi_{\mathbf{k}}\text{'s} \mid \qquad (1)$$

where N is the total number of conduction electrons in the metal. This we shall refer to as the Bloch picture. Because of the periodicity of the lattice, $\mathbf{k}$ is restricted to a certain region in k-space known as the Brillouin zone. The energy $E(\mathbf{k})$ varies continuously with $\mathbf{k}$ in each zone. Because of the exclusion principle, all the states below the Fermi level E_F are occupied by electrons. The surface in k-space :

$$E(\mathbf{k}) = E_F \qquad (2)$$

is the boundary between the occupied and unoccupied levels, and it determines many of the properties of the metal. The earliest successes of this theory include the resolution of the classical difficulty that the electrons in a metal seem to be free enough to give a high conductivity and yet contribute very much less than $\frac{3}{2}kT$ each to the specific heat. It also explains the qualitative difference between a metal and an insulator, and how semi-metals like bismuth can exist with an apparent number of 10^{-5} free electrons per atom. On this sort of picture, most of the present theory of metals is based, and Prof. Mott reminded the audience how it could account for the ferromagnetism of some metals like cobalt and nickel. However, he emphasized that in other cases we are driven to use wave functions of a qualitatively different type, namely, the Heitler–London type of function. In this picture each electron is more or less tightly bound to a particular atom (position $\mathbf{R}_n$), and moves in an orbital $\psi(\mathbf{r} - \mathbf{R}_n)$ localized around this atom. The whole wave-function is :

$$\Psi_{HL} = (N!)^{-1/2} \mid \text{determinant of } \psi(\mathbf{r} - \mathbf{R}_n)\text{'s} \mid \qquad (3)$$

In this picture the concept of a Fermi surface does not arise. Prof. Mott mentioned different kinds of ferromagnetics and anti-ferromagnetics which are thought to correspond to the two types of wave function. In particular, the ferromagnetic 3*d*-electrons in iron, and similarly the 4*f*-electrons in gadolinium metal, should be described by the Heitler–London type of wave function (3), in contrast to cobalt and nickel. This view is supported, for example, by data on the saturation magnetic moment of iron–copper alloys. The moment is just 2·22 Bohr magnetons multiplied by the number of iron atoms, in accordance with the view that each magnetic electron is localized

around an iron atom and is therefore removed when the iron atom is replaced by a non-magnetic copper atom. The behaviour is quite different from that of cobalt–copper and nickel–copper alloys. Later in the conference, these ideas were developed more fully and more experimental evidence was brought forward by the following: W. M. Lomer and W. Marshall, "Electronic Structure of Ferromagnetic Alloys"; Miss Y. Cauchois, "X-ray Spectroscopy of Metals"; B. R. Coles, "Electrical Resistivity and Electronic Structure". There was also ample time on the programme for the lively discussion which recurred throughout the conference, and in which many other people contributed. J. R. Schrieffer outlined how wave functions, which are quite different again from either (1) or (3), are being used in "Recent Developments in the Theory of Superconductivity".

All the rest of the conference was devoted to non-ferromagnetic good metals like the alkalis, and copper, aluminium and lead, and their alloys. Even for a good metal, the Bloch type of wave function (1) cannot be regarded as satisfactory, in spite of its many successes. For a free-electron gas which can be worked out in detail, it gives a zero density of states at the Fermi-level, in contrast to all the experimental evidence, which demands a finite one. As R. S. Leigh pointed out in his paper on "Correlation in the Free Electron Gas", this difficulty can be traced to the long-range ($1/r$) nature of the Coulomb potential. The use of the Bloch wave function (1) implies that each electron moves in its orbital ψ_k independently of all the other electrons. This cannot be correct, for the Coulomb repulsion between two electrons is quite strong and must influence their motion so as to keep them from getting too close to one another. This is called 'correlation'. It is closely allied to the longitudinal plasma oscillations of the electron gas as a whole, for which the Coulomb repulsion is also responsible. By starting from these plasma oscillations, a more complicated theory of the free electron gas can be built up following the work of Bohm and Pines, which gives reasonable agreement with experiment for the alkali metals. Mr. Leigh discussed how these results could also be obtained from a somewhat different point of view.

If this left the conference momentarily stunned by the complication of the theory of even a free-electron gas, despondency was soon dispelled. In spite of the complications of correlation, the wave function for the free-electron gas can be written (to a good degree of approximation):

$$\Psi = (N!)^{-1/2}\ \chi\ |\ \text{determinant of } \psi_k\text{'s}\ |\qquad (4)$$

This is very similar to (1), while the extra factor χ, called the plasma term, describes the correlation of the electrons. The determinant again describes N particles moving independently of one another through the metal. But these particles are no longer simple electrons. The Coulomb repulsion means that each electron is surrounded by a denuded region in which other electrons are unlikely to be. Because of the positive charge of the ions making up the lattice, this denuded region has on the average a positive charge. The particles now consist of the electrons together with the denuded region around each one. Each particle is therefore electrically neutral, and there is now no long-range Coulomb force between them. Only a weak short-range force remains. For this reason there is little correlation and the particles can be described as moving nearly independently.

The wave function (4) thus contains a single determinant of extended orbitals, ψ_k. This removes the deficiencies of the simple Bloch theory based on (1), and yet at the same time retains its successes. The concepts of the wave vector **k**, Brillouin zones and Fermi surfaces remain, and so does our interpretation of physical properties in terms of them. The theme was developed further by J. Hubbard in "Description of the Electron Gas in Terms of the Independent Particle Model". Using this model as a basis for the first time, V. Heine described a "Self-consistent Band Structure Calculation for Aluminium". In this calculation he was also able to overcome some of the mechanical difficulties that have always made calculations of band structure so unreliable, and to obtain reasonable agreement with what is known experimentally about the Fermi surface in this metal. W. M. Lomer presented a paper for Mrs. E. C. Ridley on the energies of the "6*s*-, 6*p*-, 7*s*-, 7*p*-, 6*d*-, 5*f*-Wave Functions of Metallic Uranium". From this the six valence electrons would appear to be predominantly in the 5*f*-band. Prof. R. Eisenschitz described how one could calculate the "Electronic Levels of Disordered Structures".

We may summarize by saying that for various experimental and theoretical reasons, some of which have been mentioned above, we believe that the concepts of Brillouin zones and Fermi surface have real validity and physical significance, in spite of the complications of electron correlation. Most of the conference was therefore devoted to methods of determining experimentally the shape of the Fermi surface in a given metal (as well as other aspects of the band structure), since this is the key to an understanding of most of the electrical and magnetic properties of the metal. The de Haas–van Alphen effect, discussed by Dr. D. Shoenberg in a paper of that title, would appear to be in principle the most powerful tool for obtaining the shape of the Fermi surface experimentally. The observations give the area of cross-section of the surface normal to the magnetic field, as a function of the field direction, and from this the shape of the surface can be calculated. If the Fermi surface consists of several pieces in different zones, then each piece can be so determined. A. Gold described how "The de Haas–van Alphen Effect in Lead" had enabled him to map out by this method the Fermi surface in lead, which consists of several pieces in the second, third and fourth zones. It is noteworthy how close the band structure is to what one obtains if the Fermi surface is taken as a sphere containing four electrons per atom (free-electron gas model) and the pieces of the sphere are fitted correctly into the zone scheme. This work made us feel that perhaps all other methods could be put in a subsidiary category; but Dr. Shoenberg reminded us that the de Haas–van Alphen effect has its 'blind spots', where it does not at present seem to be applicable. For example, the effect has not yet been detected in any alkali or noble metal. For these metals other methods must be used, and Dr. A. B. Pippard discussed "The Anomalous Skin Effect" with particular reference to determining the Fermi surface of copper. He concluded that the Fermi surface either just touches or just does not touch the zone face in the (111) direction. Circumstantial evidence would favour the former. Dr. R. G. Chambers presented a paper on "Cyclotron Resonance in Metals". The resonance could in principle determine the effective mass at all points on the Fermi surface, as in semiconductors, but some of the differences from

cyclotron resonance in semiconductors were pointed out. So far only bismuth has been studied systematically, but the resonance has also been detected in tin.

This more or less concludes the most powerful tools, in which some quantity is measured as a function of orientation for all directions in space so that much detailed information is obtained. In contrast, the following determinations give a specific piece of information, which may not be obtainable in any other way, but which is an average over the whole band structure or the whole Fermi surface. J. A. Catterall discussed "X-ray Emission from Alkali Metals" with particular reference to lithium. He concluded that the maximum in the emission spectrum definitely came below the Fermi level. Miss R. Coldwell-Horsfall presented a paper on the "Electrical Conductivity Tensor and the Fermi Surface". There was considerable discussion about the density of states at the Fermi-level and related experimental quantities such as the electronic specific heat and the Knight shift. Papers on this were presented by Prof. H. Jones ("Experimental Data Related to Band Structure"); P. L. Smith ("Electronic Specific Heat of Simple Alloys"); L. E. Drain ("Nuclear Magnetic Resonance in Ag–Cd Alloys"). Prof. Jones pointed out that the electronic specific heat measurements require a density of states about 50 per cent higher than one would expect, and gave a theory of how this might be accounted for. The results presented by P. L. Smith and L. E. Drain showed how much more difficult it is to make sense of the results for alloys, and there was considerable discussion about this.

At the end of the first day of the conference, Prof. L. C. Jackson helped to take our minds off metals when he delivered the Duddell Lecture of the Physical Society on "Measurements of the Thickness of the Helium Film". The subject of this very interesting lecture was particularly appropriate to the conference since many of the experimental methods discussed involve low-temperature techniques. V. HEINE

OBITUARY

Prof. R. W. Whytlaw-Gray, O.B.E., F.R.S.

THE death of Robert Whytlaw-Gray on January 21 at the age of eighty removes one more link with the days when the Curies and Ramsay were rejuvenating inorganic chemistry. He was a student of Lord Kelvin and then a research worker in Ramsay's laboratories, where he developed a life-long interest in the physical properties of gases and great experimental skill in their manipulation. This led him to work on molecular and atomic weights, and in 1906 while in Bonn he re-determined the atomic weight of nitrogen by gravimetric analysis of liquefied and fractionated nitric oxide and by density determination. Whytlaw-Gray then returned to University College, London, and in collaboration with Ramsay, determined the atomic weight of niton. This was an outstanding achievement. The density was determined with a modified Steel–Grant micro-balance and about 0·1 mm.³ of niton.

In 1914 he became senior chemistry master at Eton, where his steadiness of purpose enabled him to carry out a considerable amount of research. In 1915 and for long afterwards he engaged in confidential work on toxic and other smokes, their physical properties and the methods by which their effects could be countered. In the fifth Liversidge Lecture, entitled "The Process of Coagulation in Smokes", delivered before the Chemical Society in 1935, he discussed his own and other work on this subject, and in 1936, with H. S. Patterson, published a book entitled "Smokes". His expert knowledge was again utilized by the Ministry of Supply in 1939 and he formed a group of about twelve workers at the University of Leeds to study problems connected with chemical warfare.

Whytlaw-Gray had been appointed to the chair of chemistry at Leeds in 1922 in succession to Arthur Smithells. His research activity there was intense and he developed a buoyancy micro-balance of high accuracy and employed it in the determination of the atomic weights of carbon, fluorine, nitrogen, sulphur and silicon by the use of gaseous compounds of these elements. He was elected to fellowship of the Royal Society in 1928. After ten years in laboratories designed by T. E. Thorpe, he entered the new building which he, Dawson and Ingold had so successfully planned.

His regime was marked by tact, by careful consideration of the views of others (especially when he differed from them), by an informed appreciation of the needs of other scientific and technological departments and by a firm adherence to those principles which guided his personal and scientific life. On his retirement the Senate was "happy to learn that he is to continue his investigations in the Chemistry Department of which the spirit no less than the fabric owes so much to his unselfish devotion". One of his colleagues said of him: "It may be that some aspects of his character are derived from, or at least, related to the quiet detachment of the exact experimentalist —his unruffled calm to the poise and exactness of his own micro-balances".

When Whytlaw-Gray left Leeds in 1950 the University conferred upon him the degree of D.Sc. *honoris causa*. In recent years he worked at the Imperial Chemical Industries' Akers Laboratory, Welwyn, on the isothermals of xenon at low pressures and also on the normal density of the gas. The communication, with Dr. G. A. Bottomley, published in *Nature* as recently as December 7, 1957, is characteristic of his life-long struggle towards perfection in measurements of the properties of gases and vapours. Some earlier work on the deviation from ideality of vapours at low pressures was shown to be subject to significant error due to adsorption. This they reduced to a constant minimum in a re-designed differential compressibility apparatus, mainly by avoiding passage of mercury through greased stopcocks. The PV/P graphs for benzene vapour, after correction for adsorption, then deviated from straight lines by less than two parts in 10^4.

His colleagues and friends will be happy to think that he was able to continue in active work to the end of his long life. He rarely spoke about himself, but it was clear to those who knew him well that he served God 'with a quiet mind'.

FREDERICK CHALLENGER

NEWS and VIEWS

British Association : New Lecture for Children

Prof. J. Rotblat, professor of physics in the University of London, at St. Bartholomew's Hospital Medical School, will lecture on "Peaceful Uses of Atomic Energy" at Northgate Grammar School, Ipswich, on February 25. The lecture will be given in two sessions to different audiences, and will be illustrated by films and demonstrations ; there will also be visits to an atomic energy exhibition which the Atomic Energy Commission is arranging in Northgate School for the whole week. It is expected that some nine hundred children will hear the lecture, arrangements for which have been made by Mr. L. R. Missen, chief education officer, East Suffolk, in co-operation with the British Association for the Advancement of Science, as part of the new campaign of the latter to present science to the people.

Pure Mathematics at Cambridge : Prof. A. S. Besicovitch, F.R.S.

Prof. A. S. Besicovitch, Rouse Ball Professor of mathematics in the University of Cambridge, retires this year, having reached the age of sixty-seven. He was elected to the chair in 1950, succeeding J. E. Littlewood, who was the first holder. He has recently worked at a number of problems in real-function theory, in particular the theory of area and extremal problems. He has directed research in these topics but he has not restricted his teaching to graduates. To the pleasure and profit of those working for the Mathematical Tripos, he has organized seminars for them. He has also maintained a flow of 'Contest Problems', at which an aspiring young analyst may try his skill with the possible reward of a public announcement : 'Perfect solution of problem was made by *A. B. C.*'. Prof. Besicovitch has been credited with the remark that a mathematician's reputation rests on the number of bad proofs that he has given—in the sense that pioneer work can be rough. He preserves unimpaired his mastery of intricate mathematical situations, and many more good and bad proofs are expected from him.

Prof. H. Davenport, F.R.S.

Prof. Harold Davenport, who has held since 1945 the Astor chair of pure mathematics at University College, London, will succeed Prof. Besicovitch on October 1. Born in 1907, he took degrees at Manchester and at Cambridge, becoming a Fellow of Trinity College. He was elected to the Royal Society in 1940. Most of his work has been in some branch or other of the theory of numbers. His early papers dealt with problems of the analytic theory, following the methods of Hardy and Littlewood. Later he turned to Diophantine approximation and the geometry of numbers. He has written about a hundred papers and some of them give solutions of problems which had been long outstanding. To mention only one example, Minkowski's problem, of the minimum value of the product of three linear forms the determinant of which is 1, was solved with consummate skill and elegance in 1938. He has now in course of publication some fundamental results on the minima of indefinite quadratic forms. Prof. Davenport has built up a strong school of research at University College and there are mathematicians of international repute who owe their early guidance and encouragement to him. He is one of the founders of the journal *Mathematika*, and he has just been elected president of the London Mathematical Society. One of the most resourceful English pure mathematicians of his generation, he is expected to solve with distinction as difficult a problem as he has yet tackled—that of succeeding Littlewood and Besicovitch.

Aircraft-propulsion Studies at Cranfield : Mr. A. G. Smith

Mr. A. G. Smith, who has been appointed to the chair of aircraft propulsion in the College of Aeronautics, Cranfield, entered the Royal College of Science and Technology, London, as a Royal Scholar in 1937 and graduated with a first-class degree in physics in 1939. He studied aeronautics for a post-graduate year at the City and Guilds College, London, and then worked for Blackburn Aircraft, Ltd., at Brough, until 1942, when he joined Power Jets, Ltd. During the rest of the Second World War he was engaged on research and development work on various Whittle engines, and particularly on compressor aerodynamic development. He joined the National Gas Turbine Establishment on its formation and worked on theoretical and experimental aspects of turbine cooling. In 1952 he was appointed reader in the University of London in applied thermodynamics with special reference to gas turbines. Since that time, besides carrying out research on aerodynamic and heat-transfer problems, he has run a very successful postgraduate course in gas-turbine technology at the City and Guilds College. He has published papers on turbine cooling and on heat transfer, aerodynamic and thermodynamic matters related to gas turbines.

Horticulture at Nottingham : Prof. J. P. Hudson, O.B.E.

The newly created chair of horticulture at the University of Nottingham has been filled by the appointment of Dr. J. P. Hudson, the present head of the Department of Horticulture, who thus becomes the first student of the old Midland Agricultural College to succeed to a chair. Dr. Hudson has had a varied career. Entering college in 1927, he graduated with the degree of B.Sc. (Hort.) of the University of London in 1930, and then served one year as student assistant in the Agricultural Economics Department at the Midland Agricultural College. From then until the outbreak of war in 1939 he was engaged in practical horticulture, at first in industry but later as horticultural instructor at the East Sussex School of Agriculture. During the War he served with considerable distinction in the Royal Engineers and in 1942 spent six months in the United States organizing a bomb disposal school. For the last four years of the War he was engaged on bomb disposal research and experimental work, and was made O.B.E. and awarded the George Medal (and Bar) for his services. Indeed, he must be one of the few professors to discover an aptitude for research, six years after leaving the university, on a material where a decisive result might well have the rather unusual effect of terminating one's research career.

Leaving the army on the termination of hostilities with the rank of major, he proceeded to New Zealand to take charge of the Dominion Horticultural Station at Levin, where he was responsible for horticultural extension work in the market garden, glasshouse and nursery industries. He returned to England in 1948,

as lecturer in horticulture at his old institution, now become the Faculty of Agriculture of the University of Nottingham. He was appointed head of the Department of Horticulture in 1953, after being acting head for two years. Since 1951, Hudson has been steadily developing his Department from that of a college concerned largely with certificates and diploma courses into a University Department of Horticulture. The depression in the horticultural industry which led to a fall in the number of candidates coming forward for degree courses made this a difficult period for the change, but Hudson successfully piloted his Department through, initiating at the same time a vigorous research school. The theme has been the influence of cultural practices on plant growth, and his contributions in two fields, namely, the control of soil moisture for glasshouse crops, and the influence of environment on the growth of biennial crops, have shown his excellent grasp of the fundamental principles involved, and the manner in which they may be applied to the benefit of the horticultural industry.

Dean of Graduate Studies at the Rockefeller Institute, New York: Dr. Frank Brink, jun.

Dr. Frank Brink, jun., has been appointed to the newly created office of dean of graduate studies at the Rockefeller Institute. Dr. Brink is a member and professor of the Institute, doing research work on biophysics. As dean, he will assist the president and the faculty committee on educational policies in the development of graduate education. The charter of the Rockefeller Institute was amended in November 1954 so that the degrees of Ph.D. and M.D. could be granted with the authority of the Regents of the University of the State of New York. The Institute is now in its third year as a graduate university and has a limited enrolment of graduate students. Dr. Brink has been closely associated with the education programme of the Rockefeller Institute since its inception in 1954. Since 1956 he has served as secretary of the Trustees' Committee on Educational Policies under the chairmanship of Dr. Robert F. Loeb, of the Columbia University College of Physicians and Surgeons. Dr. Brink graduated from Pennsylvania State University in 1934. After a year of graduate work at the California Institute of Technology, where he received the degree of M.S., he began graduate work in biophysics at the Johnson Foundation at the University of Pennsylvania, where he received the degree of Ph.D. in 1940. He was later instructor in physiology at the Cornell University Medical College, assistant professor of biophysics at the University of Pennsylvania, and associate professor of biophysics at Johns Hopkins University. Since 1953 he has been at the Rockefeller Institute, where he has worked in the field of biophysics and neurophysiology in association with Dr. D. Bronk, the president. He is also a member of the board of editors of the *Journal of General Physiology* and is on the editorial board of the *Journal of Cellular and Comparative Physiology* and the advisory board of the *Journal of Neurochemistry*.

Baghdad Pact Nuclear Centre

The Baghdad Nuclear Training Centre was established in 1956 by the member countries of the Baghdad Pact. It is at present concentrating on the practical applications of radioisotopes to the problems of Middle Eastern countries and is organizing intensive short courses in the uses and techniques of these materials. Two instructors each from Iraq, Iran, Pakistan and Turkey were trained at Harwell during 1956, and the United Kingdom also assisted by the gift of equipment and the loan of staff to supervise, teach and promote the development of the Nuclear Training Centre until local staff can assume responsibility. The first director of the centre, Mr. W. J. Whitehouse, was seconded from the U.K. Atomic Energy Authority in 1957 with four other members of the Authority's staff to prepare the Nuclear Training Centre, supervise the installation of British equipment there and to organize and assist in running the first course.

Mr. H. A. C. McKay of the Chemistry Division, Atomic Energy Research Establishment, Harwell, has now been appointed director of the Baghdad Pact Nuclear Centre from July 1958, in succession to Mr. W. J. Whitehouse. Mr. McKay, who was born in 1913, was educated at Balliol College, Oxford, and in 1935 was the Goldsmiths' Senior Scholar at the Institute for Theoretical Physics, Copenhagen. He was Beit Memorial Fellow at the Imperial College of Science and Technology, London, in 1937, and a demonstrator at King's College, London, in 1939. He served in the Royal Navy during the Second World War and in 1947 became a senior scientific officer in the Chemistry Division at Harwell, where he was appointed group leader of the Heavy Element Group in the Chemistry Division in 1954. He has specialized in solution chemistry.

World List of Scientific Periodicals

One result of the advances and developments in science and technology in recent years is reflected in the vast number of new scientific and technical journals that have appeared, together with an increase in the output of periodical publications throughout the world. This, combined with the fact that the third edition of the "World List of Scientific Periodicals" will soon become out of print, has led to active preparation for the issue of a fourth edition. In the new edition, brought up to date to include the new scientific and technical periodicals that have appeared in the past decade, the abbreviations of titles, where necessary, will be modified to conform with the International Standards Organization Recommendation *R4* of the International Code for the Abbreviation of Titles of Periodicals. The system recommended by the Royal Society for the transliteration of Russian, Serbian and Bulgarian for bibliographic purposes will also be adopted.

Since the location of the periodical publications available for reference in libraries is of the utmost importance to research, the librarians of the 247 libraries who contributed to the last edition are being requested to participate in the new edition of this important bibliographical work of reference. They are asked to send the titles of journals not appearing in the third edition, with particulars of their holdings, and amendments or corrections to the entries of their holdings of the 46,000 titles contained in the last edition, to the Secretary, World List of Scientific Periodicals, c/o the Zoological Society of London, Regent's Park, London, N.W.1, from which office further information may be obtained.

Thermal Insulation of Houses

The Thermal Insulation (Dwellings) Bill which Mr. G. Nabarro introduced into the House of Commons on January 29, sponsored by members of both major political parties, extends the principle of the

Thermal Insulation (Industrial Buildings) Act of July 1957 to all new dwelling houses built in the United Kingdom after 1959. Mr. Nabarro said that on the evidence of the Egerton Committee's report of 1946 and of the Ridley Committee's report of 1952 we used on average five tons of coal or its equivalent per dwelling per annum, of which four tons were used for space- and water-heating services generally. He claimed that at least 25 per cent of this would be saved by proper insulation of houses. Accordingly, at an annual construction of 250,000 houses, an initial saving of 250,000 tons should be achieved, rising progressively to 5 million tons per annum at the end of twenty years. The minimum economy of £40 million per annum, at to-day's price, made investment in thermal insulation, which should not cost more than £8 on a £1,600 four-bedroom council house, well worth while.

Stars Month by Month

There are many people who have a general interest in the phenomena of the night sky and who need a simpler description of the visibility of the stars and planets than is found in the almanacs. To meet this need *The Times* produces an annual booklet. The latest edition, "The Night Sky 1958" (pp. 27. London: The Times Publishing Co., Ltd., 1958. 3*s*. net), gives charts for every month of 1958. Each chart shows the sky as it appears at 11 p.m. on the first day of the month together with the positions of the moon and planets. Each chart is accompanied by notes on the visibility of the planets and the phases of the moon for the month. There are notes on the eclipses which occur during the year, and some general explanatory notes. The booklet is well produced and the information which it contains clearly presented. The booklet is suitable not only for the general public but also for use by those schools, societies and clubs in which efforts are made to introduce young people to the phenomena of Nature.

Bishop's Stortford School Natural History Society

The value of school natural history societies and the contribution they can make to new knowledge is illustrated by the activity of the Bishop's Stortford College Natural History Society. Observations on the mistletoe by a senior boy, D. J. Cove, were noted in *Nature* (178, 779; 1956), and will now be included by the Forestry Commission in a new book on the diseases of forest trees. Besides the usual sectional reports, the latest issue of the Society's journal, *Coturnix*, contains details of studies on the larger fungi of the Bishop's Stortford district by J. W. Neal and the distribution of certain myriapods and crustaceans in organic debris during the winter months by M. Morton. Interesting observations on the reactions of certain myriapods to light, gravity, certain smells, water and oxygen were made by J. G. Pickard, who was able to show that the relatively high rate of respiration of the lithobiomorph centipede may be correlated with its observed restlessness; the low rate in the case of *Julus* with its observed sluggishness. A regional survey of the local sewage farm was made by R. G. Oakley, while P. H. King made continuous observations at Mersea Island, the centre of the oyster industry.

Extension of Primary Cell Walls

The mechanism of deposition and extension of primary cell walls has been further investigated by G. Setterfield and S. T. Bayley (*Canadian J. Bot.*, 35, 435; 1957). Walls of elongating parenchyma and epidermal cells of *Avena* coleoptiles and onion roots were studied by autoradiography, using sugars labelled with carbon-14, and by electron microscopy of thin transverse sections. The autoradiographs showed that deposition of cellulose takes place over the whole length of the cell, ruling out the possibility of bipolar tip growth. Autoradiographs of cells grown for short periods in labelled sugar gave no evidence of localized incorporation of the isotope around primary pit-fields, but rather indicated that deposition of microfibrils is finely dispersed over the entire wall surface. The thin sections revealed a marked change in wall structure as elongation proceeds. Walls of young cells contain only transversely oriented microfibrils, while older walls include an inner region of predominantly transversely oriented microfibrils bounded outside by a region with microfibrils showing an irregular transition to longitudinal orientation. The degree of longitudinal orientation in the outer region increases with length of cell. These results for thin walls are consistent with the multi-net model for wall growth deduced by other workers from electron microscope studies on cell fragments. Thin sections have also revealed layering in several types of growing walls which is difficult to interpret on the basis of the multi-net model alone.

Graft Unions between Meristematic Tissues

Under the title of "The Regeneration of Severed Pea Apices", H. F. Gulline and R. Walker (*Australian J. Bot.*, 5, 129; 1957) have described successful graft unions between meristematic tissues in apices of the pea, *Pisum sativum* L. A technique for preventing desiccation of apices during and after the operations is described. Apical segments ranging from about 200μ down to 50μ in depth were successfully grafted back to their parent plant. The subsequent development of grafted apices was completely normal. These experiments show that a complete shoot can be regenerated from an apical segment less than one-thousandth of a cubic millimetre in volume, containing about 600 cells. This is smaller than any graft previously recorded. The morphogenetic interest of this investigation needs no emphasis.

U.K. Atomic Energy Authority: Appointments

Dr. H. Kronberger has been appointed director of research and development at the Industrial Group Headquarters at Risley, Lancashire, of the United Kingdom Atomic Energy Authority. He succeeds Mr. L. Rotherham, whose appointment as a member of the Central Electricity Generating Board was announced in *Nature* of January 4, p. 20. Dr. Kronberger, who is thirty-eight years of age, was chief physicist at Risley prior to his new appointment.

Mr. J. C. C. Stewart, formerly director of technical policy in the Industrial Group at Risley, becomes a deputy managing director of the Industrial Group. Mr. J. B. W. Cunningham, formerly deputy director (civil reactors), becomes director of industrial power in the Industrial Group. Dr. R. Hurst, formerly chief chemist in the Research and Development Branch of the Industrial Group at Risley, becomes director of the Dounreay Experimental Reactor Establishment. Mr. D. S. Mitchell, formerly director of administration, becomes director of personnel and administration in the Industrial Group. These appointments, which took effect on February 1, mark a first stage towards implementing recom-

mendations made by the Committee appointed by the Prime Minister under the chairmanship of Sir Alexander Fleck to examine the organization of certain parts of the Authority. Further appointments will be announced in due course.

Mr. D. W. Fry, chief physicist at Harwell, has been appointed a deputy director of the Atomic Energy Research Establishment and took up his new post on February 17.

Prof. Heinrich Balss

Dr. J. Théodorides of the University of Paris has written to say that, in the obituary notice of Prof. Heinrich Balss, which appeared in *Nature* of December 21, 1957, p. 1391, he was surprised to find no reference to Prof. Balss's important works on the medieval naturalist, Albert the Great. Dr. Isabella Gordon wishes to add that Prof. Balss's scholarly contributions to the history of biology and medicine include the following: "Albertus Magnus als Zoologe" (Munich, 1928); "Albertus Magnus als Biologe: Werk und Ursprung", *Grosse Naturforscher*, 1 (Stuttgart, 1947) and "Aristoteles Biologische Schriften", with Greek text and German translation (Munich, 1943).

Announcements

The following medal awards have been made by the Royal Astronomical Society: *Gold Medal:* Prof. André Danjon, for his contributions to astronomical photometry, to fundamental astronomy and to the design of astronomical instruments; *Eddington Medal:* Dr. H. W. Babcock, for his work on the magnetic fields of early-type stars and of the Sun.

Dr. L. A. Jordan, director of the Research Association of British Colour and Varnish Manufacturers, has been appointed professor of chemistry at the Royal Academy of Arts in succession to Dr. H. J. Plenderleith, keeper of the Research Laboratory, British Museum.

The British Institute of Radiology has made the following awards for 1958: the Barclay Medal to Dr. G. M. Ardran (Nuffield Institute for Medical Research, Oxford); the Röntgen Award to Dr. P. R. J. Burch (Department of Medical Physics, General Infirmary, Leeds); and the Barclay Prize (posthumously) to the late Dr. Trevor Griffiths (Brook Hospital, Shooters Hill Road, London).

The Road Research Laboratory of the Department of Scientific and Industrial Research is again holding open days, this year on May 15 and 16. The Materials and Construction Division and the Colonial Section are at Harmondsworth, Middlesex, and the Traffic and Safety Division at Langley, Buckinghamshire. The Scottish Branch of the Laboratory at Thorntonhall, near Glasgow, will be open to visitors on June 4 and 5.

The third International Seaweed Symposium will be held in Galway during August 13–19. Further information can be obtained from the Secretariat, Third International Seaweed Symposium, University College, Galway, Ireland. Abstracts of communications must reach the Secretariat before April 1.

A symposium on "The Pharmacology of Phenolic Substances" is to be held at Oxford on April 10 and 11 in conjunction with the first annual general meeting of the Plant Phenolics Group. Applications to attend should be made to the secretary, Dr. T. Swain, Low Temperature Research Laboratory, Downing Street, Cambridge.

The Nutrition Society is holding a symposium on "Fish" in the Physiology Lecture Theatre, the Old Medical School, the University of Liverpool, during March 28–29. Further information can be obtained from the hon. programmes secretary of the Nutrition Society, Miss D. F. Hollingsworth, c/o Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, S.W.1.

The 1958 International Starch Convention, organized by the German Cereals Research Association, will be held at Detmold, West Germany, during April 22–24. The programme will include papers on research and analysis; starch fractions and derivatives; starch manufacture and practice. Further information can be obtained from Dr. Eric Dux, Sichel Adhesives, Ltd., Riverside Works, 6 Friars Lane, Richmond, Surrey.

A symposium on "Numerical Approximation", sponsored by the Mathematics Research Center, U.S. Army, will be held during April 20–23 at the University of Wisconsin, Madison. The topics of the symposium include linear approximation, interpolation, Tchebycheff and other extremal approximations, expansions and algorithms. Further information can be obtained from Prof. R. E. Langer, Mathematics Research Center, U.S. Army, University of Wisconsin, 1118 W. Johnson Street, Madison 6, Wisconsin.

The spring conference of the X-ray Analysis Group of the Institute of Physics will be held in the College of Science and Technology, Manchester, during April 18–19. There is no specific topic, and papers will be presented on a wide range of subjects, including optical diffraction methods, structural investigations, and X-ray diffraction and X-ray spectrographic techniques. The conference is open to all interested persons on completion of an application form to be obtained from the honorary conference secretary, Dr. C. A. Taylor, Department of Physics, College of Science and Technology, Sackville Street, Manchester 1, to whom all inquiries should be addressed.

The Silver Jubilee Symposium on "The Physical Properties of Polymers" arranged by the Plastics and Polymer Group of the Society of Chemical Industry will be devoted to the fundamental investigation of mechanical and electrical processes in polymers and the physical testing of polymers. It will be held at the William Beveridge Hall, Senate House, University of London, London, W.C.1, during April 15–17. The proceedings will be conducted under the general chairmanship of Mr. C. E. Hollis, chairman of the Group, and Dr. V. E. Yarsley, Mr. C. G. Garton and Dr. L. R. G. Treloar will take the chair for the three daily sessions. Further information can be obtained from the Assistant Secretary (Plastics and Polymer Group), Society of Chemical Industry, 14 Belgrave Square, London, S.W.1.

In the review of "Recruitment of Skilled Trades" by Lady Williams published in *Nature* of February 8, p. 373, it is stated in the first paragraph that questionnaires were sent to 9,000 firms. This is incorrect: in fact, 1,000 firms were approached, and 550 replied.

EMPLOYMENT AND WELFARE IN 1956

IN recent years British industry has seen an expanded working population with a high demand for labour and a low rate of unemployment. The annual report of the Ministry of Labour and National Service (H.M.S.O., 6*s*.) shows that this was not the case in 1956.

The total working population (which comprises those in civil employment, in H.M. Forces and the unemployed) increased only slightly, small decreases in the numbers in civil employment and in the Armed Forces being rather more than outweighed by a rise in the number of unemployed persons. There was an increase in the number of workers on short-time and some falling-off in overtime. The number of unfilled vacancies at the end of the year was lower than at the end of 1955. The post-war trend of a falling level of employment in the basic industries and a rising level in the manufacturing industries as a whole was checked.

The total working population rose by 14,000 during the year to a total of 24,087,000 at the end of the year, with a peak in November when a new post-war record total of 24,225,000 was reached. By the end of the year the number of men and women in civil employment had decreased by 64,000 to 22,989,000 and the number of H.M. Forces was 761,000, a reduction of 18,000.

There was some easing in the demand for labour during the year, affecting mainly semi-skilled and unskilled workers. This occurred mostly in the manufacturing industries although there was a small increase in employment in some sections engaged largely on exports and capital goods. Within the basic industries, however, the general decline in man-power was arrested. The number in employment in coal mining increased by more than 3,000 and in transport and communication by 9,000 ; in the gas, electricity and water supply group the labour force remained unchanged ; there was a decrease in mining (other than coal mining) and quarrying, and agriculture again suffered a substantial decline. Other industries which increased their labour force during the year were building and contracting, the distributive trades and the chemicals and allied trades.

The average number of persons registered as unemployed during 1956 was 257,000. Although this represented an increase of 25,000 compared with 1955, it was less than in any other post-war year except 1951.

The rising trend in wages continued, although the rise in prices slowed down until late in the year when a further marked rise occurred. The post-war expansion in industrial production as a whole and in the output per man was halted in 1956, the rates remaining approximately at the 1955-level. A large measure of economy in Government expenditure was part of the Government's general disinflationary policy during 1956. By the careful choice of economy measures the Ministry succeeded in making a substantial contribution to the economies while maintaining its service to industry and the public at a high standard.

The Ministry's employment exchange and appointments services continued their primary purpose of bringing together men and women seeking employment and employers requiring workers ; they also assisted in the supply of labour to the undermanned essential industries. Employers were encouraged to offer ex-regular servicemen resettlement in civil occupations, to accept older and disabled people on their merits and to expand part-time working. In spite of some easing of the shortage of labour, there was still a high average number of unfilled vacancies on the registers of the Ministry. The changes during the year in man-power demands by a number of industries, and especially the redundancies that occurred in the motor-vehicle and other manufacturing industries, made necessary a substantial measure of redeployment of labour ; in this the Ministry, helped by a number of employers who co-operated with employment exchanges by giving early notice of labour redundancies, achieved considerable success. Following a review early in the year, the Notification of Vacancies Orders, 1952, were revoked with effect from May 7, 1956, and at the same time the arrangements for giving preference to certain classes of vacancies were discontinued. Nevertheless, the number of vacancies filled remained high, the total for the year being 2,354,000.

Vocational training was provided by the Ministry for disabled persons, for ex-regular members of H.M. Forces and for other able-bodied men and women. The total numbers of new applicants for training and those completing training during the year were fewer than in 1955. Training was given, in the main, in the Ministry's Government Training Centres.

As in previous years, special services, including industrial rehabilitation and training, were provided to assist disabled persons to obtain employment. Industrial rehabilitation was provided at the Ministry's Industrial Rehabilitation Units, and about 80 per cent of the 7,800 men and women who completed courses either obtained jobs or began courses of training within three months of leaving the units.

Although there were more stoppages of work arising from industrial disputes in 1956, there were considerably fewer working days lost than in 1955 ; the total number of days lost was, in fact, the lowest since 1952. Strikes arising from labour redundancies in the motor-vehicle and ancillary industries were a new feature.

At the end of 1956, there were sixty Wages Councils and five Catering Wages Boards, the same numbers as at the end of 1955 ; one new Council was established and one abolished during the year. Arrears of wages were paid to more than 20,000 workers following action by the Wages Inspectorate.

The increasing awareness in recent years of the need for good human relations in industry was maintained in 1956 ; the incidence of labour redundancies in some parts of the country during the year emphasized both the need for and the value of such relations. The Ministry's Personnel Management Advisory Service continued to meet requests for assistance from particular firms and to co-operate with their organizations in this field ; the value of their work is completely disproportionate to the small numbers employed.

In the international labour and social field the Ministry again made a major contribution to the work of the International Labour Organization.

DEOXYRIBONUCLEIC ACID AND GENETIC MODIFICATION IN DUCKS

IT was natural that the genetic transformation of bacteria effected by the introduction of foreign deoxyribonucleic acid should lead to speculation as to whether the phenomenon could also be induced in higher forms. That similar treatment should be capable not only of altering the racial characteristics of the growing vertebrate but that such changes would also be heritable seemed one of the least likely outcomes of such an experiment.

Recently published reports by Benoit *et al.*[1] state that they have succeeded in changing the characteristics of ducks of one breed by injections of deoxyribonucleic acid from another, and that the modifications continued to be identifiable in the progeny of the treated birds.

Because of the importance that must be attached to such revolutionary claims, and in the absence, as yet, of substantive evidence from repeat experiments, the work of Benoit and his colleagues should be subjected to critical scrutiny.

In the first place it might be suggested that, even with the most meticulous records, proof of somatic transformations of the kind described (size, weight, form of head, bill colour, carriage of body and the general aspect) would be difficult to establish in this species without an appreciation of the range of variability.

This is because there is not only considerable lack of knowledge of the genetics of the duck, but at times the development of breeds depended upon predilection for improvement by the sporadic introduction of other varieties, and this has provided many so-called pure breeds with a cryptic hereditary constitution not always to be expected from their apparent origins.

Preliminary to the experiment the birds were obtained from a reputable breeder and the weekly injections commenced when the Pekin ducklings were 8 days old. Of thirty-six ducklings, twelve (3 males, 9 females) were chosen at random for injection of deoxyribonucleic acid derived from blood and testes of Khaki Campbell drakes, and the remainder kept as controls. Total amounts of deoxyribonucleic acid injected were 5 mgm. per bird for the males and 61 mgm. for the females over a period of 5 weeks and 19 weeks respectively. One male and eight females developed the pronounced somatic modifications described. The females matured normally and laid eggs which, fertilized by treated males, produced twenty-six ducklings, 73 per cent of them having atypically coloured bills, and a good number actually showing other somatic modifications.

Bill colour in the Pekin breed is orange-yellow, and in the Khaki Campbell a greenish black. It may be of significance that a defect of the Pekin listed in British Poultry Standards is "Black marks or spots on the bill".

Although considerable time would appear to have elapsed between the end of the injection period and the discovery of the modifications, the findings, if and when substantiated, would, like the proof of vegetative hybridization, have a tremendous impact on genetic theory and practice.

For the moment it would be as well to agree with Benoit and his colleagues that "Aujourd'hui, notre meilleure conclusion sera que nous n'avons aucune conclusion à vous présenter", and await the repetition and extension of this work by them and others who will, no doubt, be stimulated by the reports.

ALAN W. GREENWOOD

[1] Benoit, J., LeRoy, P., Vendrely, C., and Vendrely, R., *C.R. Acad. Sci. Paris*, **244**, 2320 (1957); **245**, 448 (1957); *La Presse Médicale*, 65 Année, No. 72, 1623 (1957).

UNION OBSERVATORY, JOHANNESBURG

THE annual report of the Union Observatory, Johannesburg, for 1956 (which appeared in October 1957) follows the usual lines, and it will be sufficient to refer to the main events during the period under consideration. The 26½-in. refractor was used with the interferometer by Dr. W. S. Finsen for the measurement of double stars and the discovery of new ones on 135 nights, and on 68 nights for colour photography of Mars. In addition, Mr. J. Churms and Dr. W. H. van den Bos used it on 68 nights for the measurement of known double stars. Messrs. J. A. Bruwer and Churms, with a number of amateur astronomers who are members of the Transvaal Branch of the Astronomical Society of South Africa, used the 9-in. refractor on 179 nights for observations of planets, variable stars, etc., the amateurs rendering valuable assistance to the staff on visiting evenings and in many other ways. The results of the occultation observations were communicated to H.M. Nautical Almanac Office.

The Franklin–Adams telescope at the Hartbeestport Annexe obtained 178 plates for minor planets and 23 plates for comets, and the resulting positions were regularly communicated to the respective central bureaux of the International Astronomical Union at Cincinnati and Copenhagen. Dr. Finsen devoted much of his leisure time to the preparation of composite enlargements from the colour films of Mars obtained during the favourable opposition of 1954 and 1956. The card catalogue of double stars south of − 19° dec. has been kept up to date, and information has been supplied to other astronomers on request.

With the 3-in. refractor sunspot counts were obtained on 281 days, the other days being overcast or unsuitable for reliable counts. The results have been regularly communicated to the Telecommunication Research Laboratory of the South African Council for Scientific and Industrial Research, the Magnetic Observatory at Hermanus and the Receiving

Station of the South African Broadcasting Corporation at Panorama.

Under "Time Services", reference is made to improvements to the control circuits of the Diesel generator and to the vibrator power supply units so that the quartz clock installation is now practically independent of mains interruptions. A new frequency divider employing cold-cathode valves was built, and the stability of other frequency dividers improved. The performance of the ring crystal over short periods was rather more erratic than had been expected, and from October onwards all the time and frequency equipment has been controlled by oscillator 44 while further tests are being made. A twelve-channel frequency recorder, designed and built by the British Post Office, was delivered in June, and this recorder has been of very great value in keeping a continuous check on the performance of all parts of the time installation. A new cathode-ray oscilloscope comparison unit was built and this measures the reception time of time signals in terms of local time to one ten-thousandth of a second.

Under "Miscellaneous Public Services" reference is made to the Wiechert horizontal seismograph which was in operation throughout the year; local earth tremors and records of ten distant earthquakes; and records of rain have been sent monthly to the Bernard Price Institute for inclusion in its monthly *Seismological Bulletin*. Reference is also made to the installation of a synchronous motor drive on the twin telescope, and to the transfer of the Leyden Southern Station from Johannesburg to Hartbeestport.

FEDERAL RESEARCH AND DEVELOPMENT EXPENDITURE IN THE UNITED STATES*

THE National Science Foundation has issued an analysis of the Federal Research and Development Budget for the fiscal years 1956, 1957 and 1958, in which latter year twenty-three agencies of the Federal Government allocated 3,400 million dollars for scientific research and development, an increase of nearly one-fourth on 1956. Actual expenditure increased from about 2,500 million dollars to almost 3,000 million dollars in 1957 and is expected to reach 3,300 million dollars in 1958. From 85 to 90 per cent of these funds are for the conduct of research and development, including the pay and allowances of personnel, actual expenditure increasing by 14 per cent in 1957, and it is expected to increase by 10 per cent in 1958 to 2,900 million dollars. Some 10–15 per cent of the total is on the expansion of plant and equipment, including the acquisition of land, and expenditure for these purposes increased from just over 200 million dollars in 1956 to an estimated 437 million dollars in 1958, mainly on plant or facilities for defence.

Of the twenty-three agencies, the Department of Defense and the Atomic Energy Commission together account for 88, 85 and 82 per cent of the total funds in 1956, 1957 and 1958, respectively, about 45 per cent of the Defense Department funds being administered by the Air Force. For 1957 and 1958, 672 million dollars were allocated by the Atomic Energy Commission, compared with 2,166 million and 2,111 million dollars, respectively, by the Defense Department. Allocations of the Department of Health, Education and Welfare for scientific research and development increased from 89 million dollars in 1956 to 200 million in 1958, and of the 182 million allocated in 1957, 175 million were primarily by the National Institutes of Health for research on the diagnosis, cause and treatment of disease. The Department of Agriculture increased its allocation from 84 million dollars in 1956 to 108 million in 1957 and 132 million dollars in 1958; for the National Advisory Committee for Aeronautics, the corresponding figures are 66 million, 80 million and 94 million dollars; for the Department of the Interior, 38 million, 47 million and 53 million dollars; for the National Science Foundation, 17 million, 48 million and 47 million dollars; and for the Department of Commerce, 18 million, 25 million and 33 million dollars, respectively, the decrease for 1958 in the allocation by the National Science Foundation being because of the International Geophysical Year.

Of the 2,231 million dollars expended on the conduct of research and development in 1956, 1,388 million was on development and 844 million on research, of which 157 million was on basic research. The corresponding figures for 1957 are estimated as 2,635 million dollars, 1,671 million, 964 million and 218 million, respectively, and for 1958, 2,782 million dollars, 1761 million, 1,021 million and 233 million, respectively. Of the expenditure on research in 1956, 198 million dollars was on the biological sciences, 616 million dollars on the physical sciences, and 30 million on the social sciences; in basic research, the corresponding distribution was 50 million, 104 million and 3 million dollars. For 1957 the corresponding distribution in total research is estimated as 281 million, 627 million and 35 million, and in basic research 70 million, 143 million and 4 million dollars; and for 1958 in total research, 317 million, 637 million and 48 million, and in basic research, 83 million, 143 million and 6 million dollars. Subdivided again, total research expenditure on the biological sciences in 1956 included 99 million dollars on medical sciences and 44 million on agricultural sciences; for 1957 the corresponding estimates are 158 million and 53 million dollars, and for 1958, 177 million and 59 million dollars. That on the physical sciences in 1956 included 6 million dollars on the mathematical sciences and 456 million dollars on the engineering sciences; corresponding estimates for 1957 are 7 million and 435 million dollars, respectively.

Of the 2,830 million dollars allocated for the conduct of research and development by the Federal Government in 1957, 48 per cent was spent on work performed by Government agencies, including pay and allowances of military personnel engaged in such work. Of the remainder, 35 per cent went to work carried out by profit organizations, about one-fourth of this going to research institutions administered by such organizations, 14 per cent went to educational institutions and the remaining 3 per cent to other institutions, including non-profit research institutions, foundations and hospitals.

* National Science Foundation. Federal Funds for Science 6: The Federal Research and Development Budget, Fiscal Years 1956, 1957 and 1958. Pp. iii+60. (Washington, D.C.: Government Printing Office, 1957.) 40 cents.

Since the 1948 low level of just over 850 million dollars, Federal Government expenditure on scientific research and development has risen steadily, except in 1954, to 2,800 million dollars in 1957, or just over 4 per cent of the total budget expenditure compared with just over 2 per cent, and it is estimated that expenditure on basic research has increased by more than 85 per cent since 1952, although still amounting to only 8 per cent of the total funds for the conduct of research and development, exclusive of the pay and allowances of military personnel. In the same period, allocations for research in the biological sciences also increased by more than 83 per cent. It is estimated that the increases have been greater than the rate of increase in costs for salaries and equipment for scientific work and that accordingly when due account is taken of such rising costs the long-term trend still reflects a steady increase in the provision of Government funds for scientific research and development. Besides the statistical tables, the report includes technical notes on the limitations of the data and definitions and a list of research centres.

PHOTOPERIODISM IN PLANTS AND ANIMALS

AN international symposium on "Photoperiodism in Plants and Animals", sponsored by the Photobiology Committee of the National Research Council of the United States, was held at Gatlinburg, Tennessee, during October 29–November 2.

In an introductory session the reversible photochemical properties of certain dyes were described by Dr. G. Oster (Polytechnic Institute of Brooklyn) and problems of energy transfer in photochemical systems were discussed by Dr. Gordon Tollin (University of California, Berkeley). Dr. Stacey French (Carnegie Institution, Stanford) discussed the difficulties of determining action and absorption spectra in living systems.

One session was devoted to the various photoperiodic effects observed in growth and dormancy of plants. Dr. J. L. Liverman (Texas Agricultural Experiment Station) and Dr. W. H. Klein (Smithsonian Institution, Washington) dealt with the interaction between red light and various other factors in the growth of bean leaf disks, and the uncoiling of the bean epicotyl hook, respectively. Both speakers reported that the effects of indole-acetic acid, kinetin, gibberellic acid and the cobaltous ion seem to be independent of those of red light. There appears, however, to be an interaction between adenine and red light in the growth of bean leaf disks. Dr. E. H. Toole (U.S. Dept. of Agriculture, Beltsville) described the responses of various light-sensitive seeds and Dr. P. F. Wareing (University of Manchester) dealt with the photoperiodic control of germination, with special reference to the action of various spectral regions and the possible role of growth inhibitors. Dr. R. J. Downs (U.S. Dept. of Agriculture, Beltsville) discussed the interaction between red and infra-red radiation in relation to various aspects of vegetative growth. The effects of red irradiation on the activity of indole-acetic acid oxidase were shown by Dr. A. W. Galston (Yale University) to be due to the formation of an inhibitor of this enzyme by red light.

Dr. R. van den Veen (Philips' Research Laboratories, Eindhoven) described experiments which involved growing various species entirely under high-intensity monochromatic light. It was found that there are marked differences between species with respect to growth responses, under the various spectral regions. There are complex interactions between blue and red, especially in relation to the control of flowering. Prof. E. C. Wassink (Agricultural University, Wageningen) also described the responses of various species grown under high-intensity radiation in narrow spectral regions.

Two sessions were devoted to the photoperiodic control of flowering. Dr. Ralph Wetmore (Harvard University) and Dr. E. M. Gifford (University of California, Davis) described the structural changes occurring in shoot-apices of *Xanthium* and other species in the transition from the vegetative to the flowering condition. The interaction of red and far-red radiation in the flowering of various species, including *Hyoscyamus*, was dealt with by Dr. H. A. Borthwick (U.S. Dept. of Agriculture, Beltsville). Dr. Roy Sachs (University of California, Los Angeles) discussed the implications of 'long-short' day plants, such as *Cestrum nocturnum*, for general theories of photoperiodism.

Dr. A. Lang (University of California, Los Angeles) read a paper dealing with the general problem of the role of auxin and gibberellins in flowering. Auxin tends to be inhibitory of flowering in short-day plants and promotive in long-day plants, but it appears only to modify the responses. Both gibberellic acid and gibberellin-like fractions from *Cucurbita* endosperm are capable of inducing flowering in some long-day plants, but cannot replace photo-induction in short-day plants. Photo-induction in long-day plants appears to involve reactions other than gibberellin-controlled processes, which may be concerned primarily with stem elongation. The interactions between gibberellic acid and photoperiodic responses were discussed by Prof. Fausto Lona (University of Parma) for a wide variety of species.

The effects of certain synthetic hormones on flowering of *Xanthium* were described by Dr. A. Naylor (Duke University), and Dr. F. Salisbury (Colorado State University) described the interaction between various applied growth substances and the critical dark period in *Xanthium*. Dr. W. Jacobs (Princeton University) discussed the relations between auxin, day-length and compensatory growth in *Coleus*.

In a general paper on the nature of the photoinductive processes in photoperiodism, Dr. James Bonner (California Institute of Technology) outlined the present state of knowledge of the various partial processes involved, and discussed the possible reasons why the 'flower-hormone' has not yet been isolated. Dr. R. B. Withrow (Smithsonian Institution, Washington) gave a general account of the kinetic aspects of the photoreactions. He distinguished between 'graded' responses (for example, of light-sensitive seeds) and 'threshold' responses (for example, photoperiodic phenomena). Dr. S. B. Hendricks (U.S. Dept. of Agriculture, Beltsville) also dealt with kinetic aspects of the photo-reactions of photoperiodism and showed how certain kinetic parameters of the photoreceptor system can be derived. He also described a second photo-receptor system which appears to be involved in anthocyanin synthesis.

A series of papers dealt with photoperiodism in animals. Dr. A. D. Lees (A.R.C. Unit of Insect Physiology, Cambridge) reviewed the phenomena of photoperiodism in insects and mites, particularly in relation to the onset of diapause. Photoperiodic responses appear to be widespread in Lepidoptera. Dr. C. E. Jenner (University of North Carolina) dealt with photoperiodism in an aquatic midge, the larval stage of which is found in the 'pitchers' of pitcher plants. Reproduction in certain snails and freshwater shrimps appears to require long-day conditions. Photoperiodism is also of importance in many marine invertebrates, including crustaceans, molluscs and echinoderms, as was shown by Dr. A. C. Giese (Stanford University).

The general problem of the seasonal control of reproductive cycles in vertebrates was discussed by Dr. W. S. Bullough (University of London), who emphasized that photoperiodism constitutes only one aspect of a highly complex system. In vertebrates, photoperiodic perception appears generally to be mediated through the eyes. In carp and related fishes, courtship and breeding appear to be induced by increasing day-length in the spring, as shown by Dr. R. W. Harrington (Florida State Board of Health). Photoperiodic effects have also been reported in certain reptiles, including the pond-turtle, the American chameleon and certain lizards. Dr. G. A. Bartholomew (University of California, Los Angeles) emphasized, however, that in the latter the importance of photoperiodic responses is probably over-ridden by behavioural patterns in relation to diurnal temperature changes.

Endogenous rhythms in the ovulation cycle of hens were described by Dr. R. M. Fraps (U.S. Dept. of Agriculture, Beltsville). Dr. D. S. Farner (State College of Washington) showed that in certain birds day-length controls a variety of physiological responses including the male gonadal cycle, fat metabolism, vernal migration and probably moulting. In the white-crowned sparrow the most effective spectral region controlling development of the testes appears to be in the red. Dr. A. Wolfson (North Western University, Evanston) described photoperiodic control of migration in the juncos of Alberta, in which fat deposition appears to be an important factor. The responses in these birds appear to be determined by the duration of the daily dark period. Photo-periodic responses in other bird species were described by W. L. Engels (University of North Carolina), Dr. C. M. Kirkpatrick (Purdue University) and Dr. W. O. Wilson (University of California, Davis).

A further series of papers was devoted to endogenous rhythms in plants and animals. Dr. E. Bünning (University of Tübingen) gave a general account of endogenous rhythms, and discussed some general properties of the 'timing mechanism' in such rhythms. Dr. K. C. Hamner (University of California, Los Angeles) described the results of experiments with 'Biloxi' soybean, in which it was found that flowering occurred with cycle-lengths of 24 hours or of multiples thereof, whereas with cycles of intermediate duration flowering was partially or completely inhibited. These results are held to indicate an endogenous rhythm in photoperiodic sensitivity. Dr. F. W. Went (California Institute of Technology) showed that certain species, notably the tomato, made favourable growth on 24-hour cycles but showed reduced growth and other adverse symptoms on other lengths of cycle. These effects are exhibited in both photoperiodic and thermoperiodic phenomena.

Dr. C. S. Pittendrigh (Princeton University) discussed the possible nature of the clock mechanism in endogenous rhythms and postulated that two types of 'oscillator' may be involved, namely: (1) a primary light-sensitive oscillator which is temperature-independent, and the phase of which is determined by external light conditions; (2) a temperature-sensitive oscillator which can 'entrain' oscillator (1). Endogenous rhythms of activity in the hamster, and of bioluminescence in *Gonyaulax polyedra*, were described by Dr. K. S. Rawson (University of Wisconsin) and Dr. J. W. Hastings (Northwestern University) respectively.

Three evening sessions were devoted to sixteen short papers, including seven dealing with interactions between gibberellic acid and various light-controlled processes.

This highly successful meeting is believed to have constituted the first international symposium devoted specifically to photoperiodism in plants and animals. Biologists working in this field are indebted to the Photobiology Committee for organizing the meeting and especially to Dr. R. B. Withrow and Dr. N. E. Tolbert, chairman and secretary of the Committee respectively.

P. F. Wareing

FARMING HISTORY

THE joint annual conference of the British Agricultural History Society and the Association of Agriculture was held on December 7 at the Institute of Education, University of London. Three papers were read. Mr. George Ordish discussed the "History of Crop Pests, and the Measures taken to overcome them". After lunch, two papers on horticultural history were presented. Dr. L. G. Bennett, Department of Agricultural Economics, University of Reading, spoke on the "History of the Development of Market Gardening", and Mr. Eric Hobbis, of Long Ashton Research Station, University of Bristol, on the "History of Soft Fruit Growing".

Pests and diseases of plants are as ancient as the cultivation of crops for food, and the uncultivated flora that flourished before man was probably also afflicted. Mr. Ordish showed by exhaustive references to Pliny that pests and diseases were well known in classical times. Apples and pears were affected, and the olive fly was prevalent. It is still a trouble. There were cereal pests, wireworm and the cabbage flea beetle, but there was no phylloxera, nor the two mildews that were introduced from America much later.

Methods of protection were then of four kinds: religious, superstitious, mechanical and chemical. Though some of the first two were peculiar, it is not advisable to dismiss them out of hand. The superstition of yesterday often turns out to be the science of to-day. Achilles barley, presumably an immune variety, was recommended by Pliny when rust was very bad. Treating with burnt ash, steeping seed in

wine and the dregs from the oil presses were other preventive measures. Greasebands were fixed on the vines, and indeed are still used in Turkey.

No doubt the prevalence of pests and diseases, and the lack of knowledge of how to destroy or cure them, was a factor, among others, that kept yields at a low level for a thousand years or more.

Mr. Ordish proceeded to a discussion of the work of the sixteenth- and seventeenth-century botanists, more particularly that of Thomas Mouffett and Topsell. He said that Worlidge in 1669 had set out a method of dealing with hop mildew that would effectively control one type. The belief in the spontaneous generation of bugs, lice and other insects "out of corruption" was widely held by writers of that date, and was not disposed of until later scientists had shown that these pests were born out of eggs. New material had become available in the eighteenth century, and Richard Weston and others suggested the use of tobacco dust, spraying with a nicotine solution, and fumigating with tobacco smoke. Steeps of brine and urine had continued to be used to control cereal rusts, but there was some dispute about their efficacy. Some remarkable randomized experiments were made by Tilly in France about 1750 to test controls.

Necessarily, Mr. Ordish's paper was an outline of a vast subject, and, as was then said, it is to be hoped that he will make and publish an exhaustive study of the subject. The only existing treatise is a very brief American publication.

Dr. Bennett traced the development of commercial market gardening from the sixteenth to the nineteenth century. Before 1500 he thinks there was little. The use of vegetables is said to have declined between the eleventh and the sixteenth centuries, and to have been revived only with the new methods of cultivation introduced to Great Britain by French and Dutch refugees from religious persecution. It is, however, a little difficult to accept this conclusion, because, if it is true, some other food must have taken the place of the onion, garlic, leek, cabbage, peas and beans, worts and other herbs that all went into the pottage commonly consumed by the generality of the people. Be that as it may, Dr. Bennett made it quite clear that there was a great extension of market gardening in the sixteenth century. The refugees settled in the Sandwich area and elsewhere in Kent, around Norwich, and eventually in the immediate environs of London, where a part of Covent Garden was set aside for a vegetable and fruit market held three days a week. The Worshipful Company of Gardeners was organized by the growers to exercise control over malpractices and to look after their interests. From this time market gardening increased in importance, especially in the nineteenth century, when the growth of population in large aggregations, some rise in the standard of living, and changes in dietetic taste led to an immensely increased demand for fruit and vegetables.

Mr. Hobbis indicated no such decline in the use of soft fruits during the Middle Ages as there may have been in vegetables and herbs. Strawberries, raspberries and gooseberries were known to our remote ancestors, but the raspberries were not very well liked until the sixteenth century. They were thought sour. Red currants were then regarded as a small kind of red gooseberry. From such small beginnings a very large soft-fruit growing industry had developed by the nineteenth century, and it expanded widely during that century, though it had not then spread to the eastern counties. The two wars had a bad effect, but the soft-fruit growing industry under modern influences—factory jam-making, canning, deep freezing, and so on—is reviving, while the results of research are providing growers with advantages unknown to their predecessors.

Such subjects are not simple, and it is difficult to make a summary of all the important points raised in these two lectures and the discussion that followed them. All three lecturers demonstrated that there are large areas in the history of agriculture and horticulture that still require exploration.

The headquarters of the British Agricultural History Society have now been moved to the Department of Agriculture, Parks Road, Oxford, and anyone interested should make inquiries of the Hon. Secretary at that address. G. E. Fussell

GELATINE AND GLUE

At the fourteenth meeting of the Research Panel of the British Gelatine and Glue Research Association, held on December 12, Dr. S. M. Partridge (Low Temperature Research Station) took the chair for the morning session and Mr. S. G. Hudson (Richard Hodgson and Sons, Ltd.) for the afternoon session. The first paper was by Mr. G. Russell (Ilford, Ltd.) with the title "Chemically Distinct Major Components of Gelatin"[1]. He outlined previous views on the level of impurities (other than inorganic ions) in high-grade gelatines. Methods such as heat coagulation, or adsorption on charcoal, suggest a protein impurity-level of not more than 0·5 per cent. In an attempt to separate these impurities from gelatin, experiments were carried out, in which chromatographic column technique was applied, using a finely divided form of the resin, '*I.R.C.*50'. Elution was effected with a buffer previously used for equilibrating the column, so that the whole operation took place at the same *p*H. Protein in the fractions was estimated, after hydrolysis, by reaction with ninhydrin.

Whereas at *p*H 6 the whole of the gelatin sample is eluted, giving a curve of concentration against eluant volume with a twin peak, at *p*H 5·5 only a portion of the sample (15 per cent) is eluted, the remainder (85 per cent) being firmly adsorbed on the resin. The latter fraction can be removed by raising the *p*H. Paper chromatograms show the smaller component to have a higher tyrosine content than ordinary gelatin, no detectable hydroxyproline, and other characteristics giving it an amino-acid composition broadly similar to the serum albumins. The major component is similar to gelatin. Quantitative results for tyrosine, using ultra-violet absorption, show the major component to have zero or only a trace of tyrosine, whereas the minor component has about 3 per cent. Assuming the tyrosine to be entirely present in the minor component, the content of this component in a series of gelatins ranges from 10 to 20 per cent. Mr. Russell considered some of the implications of his work, and a keen discussion showed the interest aroused. Dr. G. R. Tristram (University

of St. Andrews) reported a separation, presumably of the same two components, on a modified cellulose column. It was clear from many comments that the future course of research on gelatin may be very much modified by Mr. Russell's work.

The second paper, "Some Aspects of Connective Tissue in Relation to Age", by Dr. R. Consden and Dr. P. C. Brown, of the Special Unit for Juvenile Rheumatism of the Canadian Red Cross Memorial Hospital, Maidenhead, discussed the chemical make-up of connective tissue. Recent work, both on collagen and on the polysaccharides of connective tissue, has revealed the complexity of the problems requiring solution. Whereas certain polysaccharides are readily removed by very mild processes, others are so firmly held that their association with the collagen must be very intimate. This applies in particular to the neutral polysaccharides. The course of fibrogenesis was outlined, and the contrast between young tissues, whether in young animals or in freshly formed tissue in wounds, and mature tissues was emphasized. The inertness of collagenous tissue was illustrated from Glynn's experiments on embedding dead tissue in living animals to study their rate of destruction. Collagen appears to persist indefinitely, whereas liver or muscle is metabolized fairly quickly. If, however, the collagen is heat-shrunk, it then is attacked fairly rapidly. The collagen of the uterus, which Harkness has studied, shows unusual features in being able to undergo rapid growth during pregnancy and rapid reabsorption at parturition. The increased stability of collagen with age, which can be demonstrated also as an increase in the force to inhibit contraction under thermal shrinkage conditions, is related to greater orientation and crystallinity. Keech has shown that a greater resistance to collagenase develops. A survey of human collagenous tissue shows that there is a significant increase of shrinkage temperature with age, and older tissue is more resistant than young to the action of hydrogen-bond breaking reagents. Uterine collagen has a significantly lower shrinkage temperature than adult collagen from elsewhere.

The third paper, "An Approach to the Non-Gelatin Constituents of Gelatines and Glues", was given by Mr. A. A. Leach, of the Association staff. Using bone glue as the starting material, it is possible to separate from it a component, present in substantial amount, which partially coagulates at about *p*H 4 and can be centrifuged off. This component is made up of a complex of lipid, polysaccharide, non-collagenous protein and a gelatin-like substance. The lipid is derived from the bone fat, and the analytical evidence suggests that the polysaccharide and non-collagen protein have their origin in the 'osseomucoid' isolated from compact bone and analysed by Eastoe and Eastoe. It is not certain to what extent the links between gelatin and the complex are formed during processing in bone glue manufacture, or are already present in the tissue. Attempts to separate the gelatin from the complex, using either heat coagulation of the second protein or differential trypsin attack on the gelatin, although partially successful, were not quantitative.

Bone glue from which the complex had been removed was fractionated by the alcohol method of Stainsby. It was then shown that the bulk of the non-gelatin components, other than the complex, remain in solution at the end of fractionation. They include substantial amounts of polysaccharide. It must be presumed that a further separation of the 'gelatin-like' fractions into the two components of Russell above would still be possible using the resin column technique.

A. G. Ward

[1] Russell, G., *Nature*, 181, 102 (1958).

PIGMENTATION OF INTESTINAL MUSCLE IN STEATORRHŒA

By Dr. G. AUSTIN GRESHAM and J. G. CRUICKSHANK
Department of Pathology, University of Cambridge
AND
J. C. VALENTINE
Bedford Group Hospitals

INTESTINAL biopsy has proved of little value in the diagnosis of idiopathic steatorrhœa. Paulley[1] has described changes in the mucosa which cannot be regarded as specific.

The object of this communication is to report a hitherto undescribed association between a lipoprotein pigment in intestinal smooth muscle and steatorrhœa.

The material was obtained from necropsies on a variety of intestinal disorders (Table 1). Four of the cases of steatorrhœa were diagnosed in life on the basis of high content of fat in the stool, high proportion of unsplit fat, low serum calcium-level and megaloblastic anæmia. The percentage of fat absorbed was found to be abnormally low in three cases, using a 'fat balance' test. In two other cases, in which pigment was found, the diagnosis of steatorrhœa was not confirmed during life. In one of these cases a diagnosis of Whipple's disease[2] rested upon histological findings alone.

Pieces for histogical examination were fixed in 10 per cent formal–saline or in corrosive formal. Paraffin sections were prepared and stained by Harris's hæmatoxylin and eosin; preliminary treatment with Lugol's iodine and sodium thiosulphate was given to those fixed in corrosive formal.

Those sections which showed pigment in the smooth muscle of the small intestine were stained by histochemical methods. Sections were stained for protein by the tetrazo method of Danielli following the technique described by Dixon[3]. This method detects tyrosine, tryptophan and histidine groups in the protein molecule. The Feulgen method[4] for deoxyribonucleic acid and pyronin G[5] for ribonucleic acid were applied. The periodic acid–Schiff method[6] and the permanganate–paraldehyde–fuchsin method[7] were used for detecting carbohydrates. Sudan black was employed for detecting lipids.

The presence of an unusual pigment was suggested in the case of Whipple's disease by a brown coloration

Table 1. ILLUSTRATING THE TYPE OF CASE INVESTIGATED AND THE OCCURRENCE OF PIGMENT

Clinical diagnosis	No. of cases examined	Stomach	Jejunum	Ileum	Colon
Whipple's disease	1	*N.E.*	+	+	*N.E.*
Idio. steatorrhœa (3 yr.)	1	*N.E.*	+	+	+
Idio. steatorrhœa (10 yr.)	1	+	+	+	+
Idio. steatorrhœa (4 yr.)	1	*N.E.*	+	+	*N.E.*
Idio. steatorrhœa (5 yr.)	1	*N.E.*	+	*N.E.*	*N.E.*
Steatorrhœa (not proved in life)	1	*N.E.*	+	*N.E.*	*N.E.*
Reticulosarcoma	4	*N.E.*	–	–	*N.E.*
Tuberculosis	8	*N.E.*	–	–	*N.E.*
Secondary carcinoma	2	*N.E.*	–	–	–
Leukæmia	5	*N.E.*	–	–	*N.E.*
Gastroenteritis	14	–	–	–	–
Paralytic ileus	4	*N.E.*	–	–	–
Typhoid	2	*N.E.*	–	–	*N.E.*
Uræmia	1	*N.E.*	–	–	*N.E.*
Regional ileitis	3	*N.E.*	*N.E.*	–	–
Jejunal ulcers	1	*N.E.*	–	*N.E.*	*N.E.*

N.E., not examined; +, pigment present; –, no pigment present

of the muscular layers visible to the naked eye. In the five cases of steatorrhœa a delicate grey brown pigment was found in the hæmatoxylin and eosin preparations almost filling the cytoplasm of the smooth muscle cells of both the longitudinal and circular layers (Fig. 1). A remarkable feature was the total absence of pigment from the cells of the muscularis mucosæ. In the case in which stomach was examined and in the two cases in which colon was examined pigment was found in the same layers. Pigment was not found in any of the other examples of intestinal disease which were examined.

The histochemical reactions of the pigment were, in every case, the same. It stained bright red with the tetrazo method, purple with permanganate–paraldehyde–fuchsin and black with Sudan black. The findings suggested that it is a lipoprotein. No reaction was obtained with the other methods employed.

Fig. 1. Pigment in smooth muscle of jejunum. (Hæmatoxylin and eosin. × 450)

The presence of smooth-muscle pigment in cases of idiopathic steatorrhœa and Whipple's disease is of interest, first because of its diagnostic value and secondly because it may provide a clue to the etiology.

Paulley[1] saw pigmentation in the muscle coat in one case of idiopathic steatorrhœa, but did not consider it to be of any significance. Laparotomy was done in this case for subacute intestinal obstruction and consequently the full thickness of the gut was available for histological examination. He did not remark upon such pigmentation in his other cases. Further examples of idiopathic steatorrhœa need to be examined before the association of muscle pigment and the disease can be clearly established. Such an association is of little diagnostic value except in those cases in which the full thickness of gut-wall can be obtained. Since the pigment was not seen in the muscularis mucosæ, intestinal biopsy by Wood's tube[2] would be of little value since mucosa and muscularis only can be taken.

The histochemical properties of this pigment closely resemble those of lipofuscin (wear and tear pigment) commonly seen in cardiac muscle and in the olivary and dentate neurones. The pigment in the latter differs only in that it stains with paraldehyde-fuchsin without previous oxidation with permanganate. Scott and Clayton[9] consider that this property indicates the presence of 'highly sulphated mucopolysaccharides'. Staining with paraldehyde-fuchsin following permanganate oxidation was a feature of the pigment in the small gut and of brown atrophy pigment in the heart. This property, when the periodic acid–Schiff test is negative, suggests the presence of either cystine or highly sulphated mucopolysaccharides.

The origin of lipofuscin is debatable[10]. Marinesco[11] considers that it may arise from mitochondrial remnants. This would only seem likely if the ribonucleic acid content of mitochondria had previously been destroyed, since lipofuscin does not stain with pyronin *G*, which is considered to be a specific stain for this nucleic acid[12].

It is tempting to suppose a mitochondrial origin for the pigment in the intestinal smooth muscle in cases of steatorrhœa. Mitochondrial surfaces are sites of enzymic activity[13] which may be concerned with the absorption of fat through the intestinal wall. This hypothesis would seem more probable if the pigment was situated in the mucosa rather than in the muscle. However, the mechanism of fat absorption is still obscure[14] and such a hypothesis is worthy of consideration.

We are grateful to Dr. A. M. Barrett for his encouragement and permission to use the necropsy reports and material.

[1] Paulley, J. W., *Brit. Med. J.*, ii, 1318 (1954).
[2] Whipple, G. H., *Johns Hopk. Hosp. Bull.*, **18**, 382 (1907).
[3] Dixon, K. C., *J. Physiol.*, **120**, 267 (1953); *J. Path. Bact.*, **66**, 251 (1953).
[4] Pearse, A. G. C., "Histochemistry", 104, 425 (Churchill, 1954).
[5] Dixon, K. C., *J. Path. Bact.*, **69**, 251 (1955).
[6] McManus, J. F. A., *Stain. Tech.*, **23**, 99 (1948).
[7] Gomori, G., *Amer. J. Clin. Path.*, **20**, 665 (1950).
[8] Baird, I. McL., and Dodge, D. G., *Quart. J. Med.*, **26**, 393 (1957).
[9] Scott, H. R., and Clayton, B. P., *J. Histochem. and Cytochem.*, **1**, 336 (1953).
[10] Dixon, K. C., *J. Path. Bact.*, **63**, 85 (1954).
[11] Marinesco, G., and Tupa, A., *C.R. Soc. Biol.*, **87**, 292 (1922).
[12] Brachet, J., *C.R. Soc. Biol.*, **133**, 88 (1940).
[13] Cowdry, E. V., "General Cytology" (Hoeber, New York, 1924).
[14] Friedlander, P. H., *Brit. J. Clin. Pract.*, **11**, 516 (1957).

ENZYMIC SYNTHESIS OF COENZYME I IN RELATION TO CHEMICAL CONTROL OF CELL GROWTH

By Prof. R. K. MORTON

Department of Agricultural Chemistry, Waite Agricultural Research Institute, University of Adelaide

IT is generally accepted that the cell nucleus carries the genetic determinants of cell behaviour. It must therefore exercise control of cell division, growth and differentiation, probably by means of a compound, or compounds, synthesized exclusively in the nucleus but essential for cytoplasmic reactions. Such a compound is coenzyme I (nicotinamide adenine dinucleotide, or so-called 'diphosphopyridine nucleotide'). It is required for more enzymic reactions than any other known coenzyme and is as essential for the aerobic and anaerobic mechanism for provision of energy for the cell as adenosine triphosphate.

In animal cells, coenzyme I is synthesized from nicotinamide, according to the following reactions which are catalysed by the enzymes nicotinamide mononucleotide–pyrophosphorylase (equation 1)[1], and coenzyme I–diphosphopyridine nucleotide–pyrophosphorylase (equation 2)[2,3].

1. Nicotinamide + 5-phosphoribo-1-pyrophosphate ⇌ nicotinamide mononucleotide + inorganic pyrophosphate

2. Nicotinamide mononucleotide + adenosine triphosphate ⇌ nicotinamide adenine dinucleotide + inorganic pyrophosphate

By a tissue-fractionation method, Hogeboom and Schneider[4] showed that coenzyme I–pyrophosphorylase was mostly localized in the cell nucleus in mouse liver. Branster and Morton[5] confirmed and extended this important finding by showing that the activity per nucleus is the same in isolated nuclei as in isolated intact liver cells[6,7]. The enzyme is also found in the nucleus in normal and tumour cells from mouse mammary gland[8] and in avian erythrocytes[9]. As yet it is the only enzyme known to occur exclusively in the cell nucleus.

The coenzyme I produced in the nucleus, where it may occur in relatively high concentration[10], is supplied to the cytoplasm wherein it participates in the reactions catalysed by numerous dehydrogenases. However, it also may be degraded relatively rapidly by cytoplasmic nucleosidases[11] and pyrophosphatases[12]. Provided that the dietary vitamin (nicotinamide or nicotinic acid) intake is adequate, therefore, the amount of coenzyme I available for cytoplasmic dehydrogenation reactions depends on the relative rates of the synthetic reactions (equations 1 and 2) as compared with the degradative reactions. Synthesis of new compounds by exchange reactions *in vivo*[13] and studies with intact whole cells[8] established that degradation *in vivo* is rapid and thus there must be a relatively high turnover-rate of coenzyme I in the cell.

Branster and Morton[8] therefore tested the hypothesis that a sufficient change in the rate of synthesis of coenzyme I by the nucleus would modify normal cell division and differentiation and thus lead to malignant growth. It was assumed that the activity of the coenzyme I–pyrophosphorylase (equation 2) would limit synthesis. In comparative studies, a unit of activity was taken as the synthesis from nicotinamide mononucleotide of 1 μmole of coenzyme I per hr. per 10^9 nuclei at 38°. With C_3H mice they found that the activity of spontaneous mammary gland carcinoma (2·8 units) was about 20 per cent of that of normal lactating mammary gland (13 units)[8]. With strain *A* mice, the activity of hepatoma (9 units) induced by feeding aminoazotoluene was about 20 per cent of that of normal liver (42 units) of control animals[5]. Very low rates of synthesis were also found[5] with Ehrlich ascite cells (2 units) from mice, and with post-operative human tumours. In the latter cases, however, no suitable normal tissues were available for comparison.

Branster and Morton[8] also found that the activity of normal liver of C_3H mice markedly increases during growth, being 2 units for fœtal, 12 units for young (7 and 17 days old) and 42 units for adult mice. Thus both tumour cells, and rapidly growing fœtal cells, have low activities as compared with suitable normal cells. On the basis of the rate of synthesis of coenzyme I per nucleus, therefore, the tumour cell represents a change from an adult-type to an embryonic-type of cell, and this is consistent with the general morphological and behavioural characteristics of tumour cells.

A low content of coenzyme I (and of coenzyme II) in tumour as compared with normal tissue has been established[14,15]. When expressed as μgm. of coenzyme per gm. of tissue, dye-induced hepatomas contain 48 per cent of the total coenzyme I and 14 per cent of the total coenzyme II of normal rat liver[15]. Hepatoma and normal rat liver contain the same amount of deoxyribonucleic acid phosphorus per nucleus[16] but 546 and 249 μgm. of deoxyribonucleic acid phosphorus per gm. of tissue, respectively[17]. Thus there are about 2·2 times as many nuclei per gm. of tissue in hepatoma as compared with normal liver. Expressed on the preferable basis of amount per nucleus[8,18,19], therefore, the results of Glock and McLean[15] indicate that hepatomas contain about 20 per cent of the coenzyme I of normal rat liver. Thus there is good agreement between the relative amounts of coenzyme I and the relative coenzyme I–pyrophosphorylase activities in hepatoma and normal liver. Also in agreement with the finding of a similar amount of coenzyme I in the two tissues[15], there was no significant difference between the coenzyme I–pyrophosphorylase activity of liver in hepatoma-bearing mice and in the controls[5]. The low content of coenzymes I and II cannot be attributed to increased cytoplasmic degradation which is no greater[20], and frequently less[5], than that of comparable normal tissues. A decreased activity of nicotinamide mononucleotide–pyrophosphorylase (equation 1) may occur in tumours and thus potentiate the effect of a decreased activity of coenzyme I–pyrophosphorylase. It appears, however, that this latter effect largely accounts for the low content of coenzyme I in tumours as compared with normal tissues. A low coenzyme I–pyrophosphorylase activity would also account for the synthesis of only 25 per cent of the coenzyme I in regenerating (48 hr.), as compared with normal,

mouse liver in animals injected with large amounts of nicotinamide[21].

Weinhouse and his colleagues found that coenzyme I is a limiting factor in the respiration of homogenates and isolated mitochondria from tumours. This 'coenzyme I-effect'[22] would be expected from the low content of coenzyme I in tumour mitochondria[15], but it also may be due to the rapid dissociation of the so-called 'bound' coenzyme I from the tumour mitochondria and consequent rapid hydrolysis of the coenzyme[23]. Structural imperfection of the tumour mitochondria[24] could account for this rapid loss of coenzyme I, especially since a pronounced requirement for coenzyme I may also be shown by damaged mitochondria from normal tissues[22]. Swelling and consequent damage to isolated respiring liver mitochondria is prevented and even reversed by addition of adenosine triphosphate and coenzyme I[25], and it is likely, therefore, that the addition of coenzyme I prevents similar changes in isolated tumour mitochondria.

The 'coenzyme I-effect' observed by Weinhouse in the respiration of tumour mitochondria, therefore, reflects the low rate of coenzyme I synthesis in the tumour cell. Furthermore, the high fermentative activity of tumour cells[26,27] and of embryonic cells[22] may also be a consequence of the low rate of coenzyme I synthesis causing a kinetic imbalance of cytoplasmic enzyme systems and thus structural abnormalities in the cytoplasm.

Modification of the Rate of Synthesis of Coenzyme I as a Basis for Chemotherapy of Cancer

The findings already discussed show that in rapidly growing cells synthesis of new cell material occurs against a limiting rate of supply of coenzyme I from the nucleus. Continued cell growth immediately after cell division will cause the concentration of cytoplasmic coenzyme I to fall. It is assumed that when this declines to some critically low level, consequent interaction between the nucleus and cytoplasm causes the cells to divide again, thus providing more nuclear enzyme in relation to the amount of cytoplasm. The interaction which induces division probably involves a 'feed-back'[28] mechanism, since the synthesis of adenosine triphosphate in the cytoplasm is dependent on the supply of coenzyme I from the nucleus (for oxidative reactions), and synthesis of coenzyme I in the nucleus on supply of adenosine triphosphate from the cytoplasm. If each cell division leads to a net increase of coenzyme I–pyrophosphorylase in the nucleus, the concentration of coenzyme I will increase and thus permit of greater production of cytoplasm before another cell division is induced. In normal embryonic tissues which have no permanent lesion of coenzyme I synthesis, this will lead to a continual decline with time in the rate of cell division and so permit of normal differentiation and stabilization of the adult tissue. This interpretation is consistent with observations on fœtal, young, and adult mouse liver[8] and on regenerating mouse liver[21].

However, if there is a genetic lesion which prevents an increase in the activity of the coenzyme I-synthesizing enzyme, then cell division will not correct the limitation in the supply of coenzyme I, and synthesis of new cell material will rapidly lead to the interaction between the nucleus and cytoplasm which induces renewed cell division. The cells so formed would have the relatively high ratio of nuclear volume to cytoplasmic volume characteristic of many tumours, and cell division would remain uncontrolled. It is therefore considered that tumour cells differ from normal embryonic cells in lacking the inherited potential for increasing the amount of coenzyme I–pyrophosphorylase in response to the stimulus of cell division. Tumour cells may arise from normal adult cells by depletion of the enzyme system associated with synthesis of coenzyme I–pyrophosphorylase. Other factors which follow this primary defect may account for the invasiveness and other distinguishing features of malignant tissues.

Since the activity of the coenzyme I–pyrophosphorylase is very low in the tumour as compared with the normal adult cell[5,8], and is probably close to the critical level for cell survival, inhibition of this enzymic activity would probably be lethal to the tumour cell. However, the much greater activity (about five-fold) in the comparable normal cell ensures that a similar percentage inhibition would probably have little serious effect on the metabolism of the normal tissue, especially if the inhibition is relieved soon after death of the malignant cells. For chemotherapy, the characteristics required of the compound are as follows. (1) It should specifically inhibit the coenzyme I–pyrophosphorylase. (2) Desirably, it should be taken up preferentially by the tumour cells, which may have surface properties differing from those of normal adult cells[29]. Moreover, it should not be excluded by nuclear or cellular membranes. (3) It should not react with other enzyme systems which may modify or destroy it.

No organic inhibitors of the coenzyme I–pyrophosphorylase have yet been described. The anti-tumour agent, acetyl podophyllotoxin ω-pyridinium chloride, apparently causes a marked fall in this activity of tumour tissue in treated animals, but has no effect on the tissue *in vitro*[30]. In this Department, therefore, inhibitors are being sought which will interfere with the area of attachment of nicotinamine mononucleotide to the enzyme (see equation 2), since compounds which would react with other sites would probably inhibit also other essential pyrophosphorylases. Pyridinium compounds are being studied initially with purified enzyme from pig liver[31]. It is hoped to be able to reduce the number of compounds to be synthesized and evaluated by prediction of the relative effectiveness of substituents on the pyridine ring[32].

The powerful growth inhibitor and anti-leukæmic agent 2 : 4 : 6-triethyleneimino-1 : 3 : 5-triazine causes a marked decrease of coenzyme I in tumour cells, apparently by accelerating degradation rather than by inhibiting synthesis[33]. This and similar compounds may therefore be valuable for potentiating the action of an inhibitor of coenzyme I–pyrophosphorylase. As a long-range approach, investigation of the synthesis of the nuclear coenzyme I–pyrophosphorylase may also be of value in that activation of this system could possibly lead to production of sufficient coenzyme I to prevent division of tumour cells.

This new approach involving specific inhibitors and activators of coenzyme I synthesis would appear to deserve consideration alongside other biochemical approaches to cancer chemotherapy which were recently discussed in *Nature*[34]. The malignant cell has been cited here as a special example of uncontrolled growth. However, it is believed that active compounds of the type envisaged here may have wide application for control of growth and differentiation of normal cells, especially of plants. The problem of chemical control of coenzyme I synthesis is therefore being actively investigated in this Department.

Most of the ideas expressed here were formulated while I was on the staff of the Department of Biochemistry of the University of Melbourne, where in 1953 I initiated a programme of investigation of the biochemistry of tumours. I wish to thank Prof. V. M. Trikojus, professor of biochemistry, and Prof. S. Sunderland, dean of the faculty of medicine, University of Melbourne, for their encouragement. I am grateful to the Anti-Cancer Council of Victoria for financial assistance for the work carried out with the collaboration of Miss M. Branster.

[1] Preiss, J., and Handler, P., *J. Biol. Chem.*, **225**, 759 (1957).
[2] Kornberg, A., *J. Biol. Chem.*, **182**, 779 (1950).
[3] Kornberg, A., in "Phosphorus Metabolism", **1**, 392 (edit. by McElroy, W. D., and Glass, B., The Johns Hopkins Press, Baltimore, 1951).
[4] Hogeboom, G. H., and Schneider, W. C., *J. Biol. Chem.*, **197**, 611 (1952).
[5] Branster, M. J., and Morton, R. K. (unpublished work).
[6] Branster, M. J., and Morton, R. K., *Austral. J. Sci.*, **19**, 72 (1956).
[7] Branster, M. J., and Morton, R. K., *Nature*, **180**, 1283 (1957).
[8] Branster, M. J., and Morton, R. K., *Biochem. J.*, **63**, 640 (1956).
[9] Malkin, A., and Denstedt, O. F., *Canad. J. Biochem. and Physiol.*, **34**, 130 (1956).
[10] Stern, H., and Mirsky, A. E., *J. Gen. Physiol.*, **36**, 181 (1952).
[11] Mann, P. J. G., and Quastel, J. H., *Biochem. J.*, **35**, 502 (1941).
[12] Kornberg, A., and Pricer, W. E., *J. Biol. Chem.*, **182**, 763 (1950).
[13] Kaplan, N. O., Goldin, A., Humphreys, S. R., Ciotti, M. M., and Venditti, J. M., *Science*, **120**, 437 (1954).
[14] Jedeikin, L. A., and Weinhouse, S., *J. Biol. Chem.* **213**, 271 (1955).
[15] Glock, G. E., and McLean, P., *Biochem. J.*, **65**, 413 (1957).
[16] Davidson, J. N., and Leslie, I., *Cancer Res.*, **10**, 587 (1950).
[17] Price, J. M., Miller, J. A., Miller, E. C., and Weber, G. M., *Cancer Res.*, **9**, 96 (1949).
[18] Fishman, W. H., in "The Physiopathology of Cancer", 595 (edit. by Homburger, F., and Fishman, W. H., Paul B. Hoeber Press, New York, 1953).
[19] Leslie, I., Fulton, W. C., and Sinclair, R., *Biochim. Biophys. Acta*, **24**, 365 (1957).
[20] Quastel, J. H., and Zatman, L. J., *Biochim. Biophys. Acta*, **10**, 256 (1953).
[21] de Burgh, P. M., *Austral. J. Sci.*, **20**, 86 (1957).
[22] Weinhouse, S., in "Advances in Cancer Research", **3**, 269 (Academic Press, New York, 1955).
[23] Emmelot, P., and Brombacher, P. J., *Biochim. Biophys. Acta*, **22**, 487 (1956).
[24] Howatson, A. F., and Ham, A. W., *Cancer Res.*, **15**, 62 (1955).
[25] Price, C. A., Fonnesu, A., and Davies, R. E., *Biochem. J.*, **64**, 754 (1956).
[26] Warburg, O., "The Metabolism of Tumours" (Constable, London, 1930).
[27] Warburg, O., *Science*, **123**, 309 (1956).
[28] Umbreit, W. W., *J. Cell. and Comp. Physiol.*, Supp. 1, **41**, 39 (1953).
[29] Ambrose, E. J., James, A. M., and Lowick, J. H. B., *Nature*, **177**, 576 (1956).
[30] Waravdekar, V. S., Powers, O. H., and Leiter, J., *J. Nat. Cancer Inst.*, **17**, 145 (1956).
[31] Atkinson, M. R., and Morton, R. K. (unpublished work).
[32] Atkinson, M. R., Chem. Soc. Special Publication, Heterocyclic Chemistry Symposium, Canberra (in the press).
[33] Roitt, I. M., *Biochem. J.*, **63**, 300 (1956).
[34] Skipper, H. E., Heidelberger, C., and Welch, A. D., *Nature*, **179**, 1159 (1957).

A REMARKABLE SOLAR RADIO EVENT

By STAFF OF THE IONOSPHERE AND RADIO ASTRONOMY SECTION OF THE NETHERLANDS TELECOMMUNICATIONS SERVICES, THE HAGUE

ON November 4, 1957, a large increase in solar radio noise was observed on a frequency of 200 Mc./s. at the receiving station 'Nera' of the Netherlands Telecommunications Services. The increase started rather abruptly at 0848 U.T. and lasted for more than 5 hr., the greatest intensity being reached at about 0930 U.T., when it amounted to approximately 900 times the noise level of the quiet Sun. The smoothed intensity level showed considerable fluctuations with periods of the order of a few minutes. No solar flare has been reported as occurring near the onset of the radio event, nor was a sudden ionospheric disturbance observed. The phenomenon manifested itself over a considerable frequency-range in the metre wave-length band. It was also observed at 169 Mc./s. by the observatories at Humain (Belgium) and Nançay (France), but it was altogether absent on the decimetre–centimetre wave-length range and on a wave-length near 6 metres. The radiation was 100 per cent left-handed circularly polarized. The position of its source, as determined at 1125 U.T. with a two-element interferometer operating on a frequency of 250 Mc./s., was about 0·1 solar radius east of the centre of the solar disk. During a period of 2 hr., centred around local noon, no systematic displacement of the source of enhanced radiation was observed.

The lack of correlation with a solar flare or associated effects, and the absence of disturbances on the decimetre–centimetre wave-lengths, must be considered as rather exceptional for so large a 200-Mc./s. event. However, the extraordinary character of this great radio phenomenon was particularly apparent from records obtained with a negligible time-constant. Some were recorded with a high-speed recording paper (5 mm./sec.). These records revealed short-period fluctuations of intensity, with a period of some 0·2–0·3 sec., of a type not observed before at the Nera Observatory, altogether different from the kind ordinarily occurring during a noise storm. Part of this variability, strongly reminiscent of the ionospheric scintillations of radio sources (Fig. 1, 1), showed an oscillatory type of intensity fluctuation (Fig. 1, 2). In other parts of the record there was a preponderance of decreases in intensity, short dips of a fading character, which were also present on records obtained by the Nançay Observatory on 169 Mc./s. (for copies of which we are indebted to M. A. Boischot). During the later stages of the radio event dips and oscillations of a longer duration, of the order of 0·1 min., occurred. These could also be identified on slow-speed records obtained at 200 Mc./s. simultaneously at Nera and at Paramaribo (Surinam) with time-constants of, respectively, 1 sec. and 0·3 sec. This proves that the latter variability (which was also of a very uncommon type) certainly cannot be ascribed to ionospheric effects. No ionospheric scintillation of radio sources of any importance was observed at the Jodrell Bank Experimental Station or at the Mullard Radio Astronomy Observatory on November 4. Ionospheric scintillations of so short a period have never, in fact, been observed in Cambridge (Dr. A. Hewish, private communication). The very short-period fluctuations of intensity must also, therefore, be ascribed to solar conditions.

All these facts together justify the conclusion that the radio phenomenon which occurred on November 4 is of a new, so far unknown type of great rarity. It should be noted that this peculiar type of variability is revealed only by a recording instrument

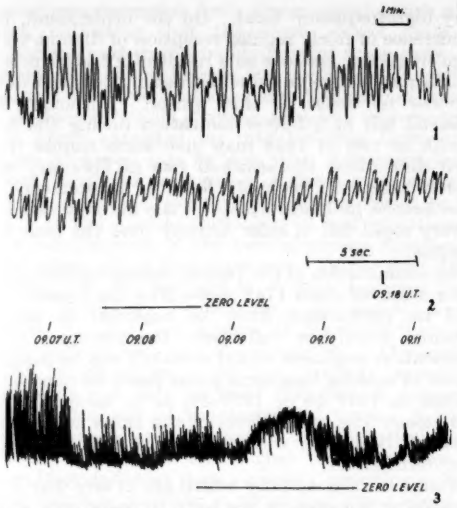


Fig. 1. 1. Scintillations of the radio source Cas *A* on 200 Mc./s.
2. High-speed record of solar radio emission on 200 Mc./s.
3. Slow-speed record of solar radio emission on 200 Mc./s.

with a time-constant no greater than a very small fraction of a second. With slow-speed recording paper one then simply obtains a very blurred record (see Fig. 1, 3), which might suggest some kind of interference. In the present case, however, the possibility of interference is ruled out, both by the confirmation from the other observatories, and by the regular pattern of the interferometer curve. The true nature of the intensity fluctuations can only be ascertained by high-speed recording. This is perhaps the reason, together with its extreme rarity, why this type of radio event has not been reported before. It differs from the so-called 'non-selective fading', observed by Payne-Scott and Little[1] on 62 and 98 Mc./s., in that the intensity fluctuations have a much shorter period.

The occurrence of fluctuations in intensity of the type shown in Fig. 1, 1, suggests that, in special circumstances, solar coronal gas may give rise to scintillation effects, possibly analogous in mechanism to ionospheric scintillations, but more varied in character. Irregularities in the solar corona may be responsible for focusing and defocusing effects and perhaps to absorption effects. It is of interest to consider the possibility that the occurrence of storm-bursts during an ordinary noise storm may be due to a focusing mechanism.

The radio event of November 4 occurred quite unexpectedly. The importance of observing such rare phenomena as completely as possible makes it very desirable that solar radio instruments should be operated on a routine basis, however specialized in type.

We are indebted to Dr. R. Coutrez of the Humain Observatory and to Dr. Boischot of the Nançay Observatory for providing us with copies of their records; and to the Jodrell Bank and Mullard Radio Observatories for information on the scintillation of radio sources on November 4.

[1] Payne-Scott and Little, *Austral. J. Sci. Res.*, A, **5**, 32 (1952).

SOLAR ACTIVITY AND THE IONOSPHERE

By C. M. MINNIS

Department of Scientific and Industrial Research, Radio Research Station, Slough

THE recent unprecedented high levels of solar activity, as indicated by sunspot numbers and described by Dr. M. A. Ellison[1], have been accompanied by equally exceptional conditions in the ionosphere. During the sunspot cycle there is no appreciable change in the amount of visible light or heat radiation emitted by the Sun, but in other parts of the spectrum this is no longer true. As compared with June 1954, when solar activity was at a very low level, the intensity of the ultra-violet and X-radiation which is responsible for the existence of the *E*-layer of the ionosphere had increased by a factor of 3·2 in September 1957, 3·6 in October and 3·1 in November. These values all exceed the previous maximum of 3·0 in May 1948 and are the highest recorded values since ionospheric measurements were begun at Slough in 1931.

The figures quoted above are based on the critical frequency of the *E*-layer. Unfortunately there is, as yet, no adequate theory on which similar estimates of the intensity of the *F*2-layer ionizing radiation could be based. However, in November 1957 at Slough, the mean value of the electron density at the peak of the *F*2-layer rose to $2{\cdot}6 \times 10^6$ cm.$^{-3}$, a value which exceeds the previous highest value, for November 1947, by 9 per cent. In October 1957 the density was much lower than in November but, after making an appropriate allowance for the normal seasonal changes in the *F*2-layer, it may be concluded that the mean intensity of the ionizing radiation in October exceeded that in November, thus confirming the conclusion reached using the *E*-layer results.

The large changes in the intensity of the ionizing radiation which are associated with the sunspot cycle lead to corresponding variations in the structure and the electron density of the layers of the ionosphere. The complicated ways in which these layers change are of great importance during investigations into the chemical constituents and the atomic processes which determine the electrical properties of the upper atmosphere. These changes are not, however, solely of academic interest, because the electron density controls the critical frequencies of the layers which are responsible for the reflexion of radio waves; a wave which is normally incident on an ionospheric layer will not be reflected for frequencies greater than the critical frequency. At very oblique angles of incidence, the greatest frequency at which a wave will be reflected will be 3–3·5 times the critical frequency for reflexion at the *F*2-layer and 5 times for the *E*-layer.

Reports from the B.B.C. receiving station at Tatsfield show that during October 1957 radio stations in the United States could, on the average, be received at frequencies of 37·5 Mc./s. in the early afternoon; in November this figure had risen to 46·9 Mc./s. On individual days even higher frequencies could be received: 50·7 Mc./s. in October and 55·3 Mc./s. in November. These observations are consistent with the reception, when propagation conditions are favourable, of B.B.C. television signals on 45 Mc./s. in the United States and in South Africa. An even more spectacular achievement, which has recently been reported in the Press, was the reception of B.B.C. television pictures in Australia at the end of November.

The mean value of the critical frequency of the *F*2-layer at Slough at noon was 13·4 Mc./s. in October and 14·6 Mc./s. in November; on individual days, values of more than 16 Mc./s. were fairly common. In the light of these figures, it is not difficult to account for reports, such as those mentioned above, of the propagation of radio waves in the very-high-frequency band over distances far in excess of those expected in normal circumstances.

It may be recalled that even near sunspot minimum in 1954 it was not uncommon to find instances where European radio stations operating in the very-high-frequency band interfered with television and other radio services in southern England. The propagation in such circumstances must be attributed, not to reflexions from the *F*2-layer, but from the sporadic *E*-layer or, alternatively, to effects associated with the refractive properties of the troposphere during certain meteorological conditions. Fortunately, such interference is not sufficiently frequent to cause serious difficulties in planning radio networks in the very-high-frequency band. On the other hand, the occurrence of fairly regular reception of distant very-high-frequency stations as a result of *F*2-layer propagation may prove to be embarrassing to some radio services for most of this winter. Although the seasonal fall in *F*2-layer ionization during the first month or two of 1958 may give some respite from such difficulties, the seasonal rise in February and March 1958 will probably lead to a recurrence of interference problems except in the unlikely event of a very rapid fall in solar activity over the next few months.

An examination of the twenty sunspot cycles which have occurred since 1749 shows that the present one and its predecessor must be regarded as having maxima which are well above the average. Communication engineers would certainly not be justified either in making long-term plans based on experience gained in 1947–48 or 1957–58, or in assuming that the steady rise in the level of the three consecutive maxima beginning with that of 1927 is part of a long-term trend.

Peaks in solar activity which are in any way comparable to the present one seem to occur only about once in a hundred years. Such an event may produce problems for radio engineers, but from the point of view of those engaged in upper atmosphere research, it may be said of the fortunate coincidence of the 1957–58 peak with the I.G.Y.: "never had mortal man such opportunity . . .".

The work described above was carried out as part of the programme of the Radio Research Board, and is published by permission of the Director of Radio Research of the Department of Scientific and Industrial Research.

[1] Ellison, M. A., *Nature*, **180**, 1173 (1957).

FOSSIL ALGAE IN INDIA

By Prof. L. RAMA RAO
Bangalore

TWENTY-FIVE years ago, I reported in *Nature* the discovery of abundant fossil algae in the Cretaceous rocks of South India, especially in one of the limestones belonging to the youngest division, the Niniyur group (Danian), of the Trichinopoly area. (The Cretaceous of the Trichinopoly Dt., South India, is divided into four groups. Starting from the oldest, these are: (*a*) the Utatur; (*b*) the Garudamangalam (Trichinopoly); (*c*) the Ariyalur; (*d*) the Niniyur. The entire succession ranges in age from the upper Albian to the Danian of the European stratigraphical scale. A full description of these rocks has been recently given by me elsewhere[1].) Further examination of all the limestones of this group and the associated flints and cherts which are their silicified representatives yielded a rich harvest of fossil algae of various kinds; these were studied and described by me in collaboration with Dr. Julius Pia, of Vienna[2]. Prior to this discovery, our knowledge of fossil algae in India was confined to casual references and brief descriptions of a few stray and solitary occurrences here and there.

The finding for the first time in India of a rich assemblage of fossil algae in the beds of the Niniyur group was therefore an event of considerable interest, and the fact that this collection included several new genera and species of the three main families, Solenoporaceae, Corallinaceae and Dasycladaceae, made it particularly noteworthy. Many of these forms show features of significant phylogenetic and evolutionary importance, the entire collection constituting, according to Pia, "the most interesting algal flora" he had ever studied.

The publication of the memoir in 1936 dealing with the Niniyur algae thus opened out a whole field of research, and it is gratifying to note that many valuable contributions to our knowledge of fossil algae in India, especially from the Cretaceous and Eocene rocks, have since been made by a number of workers. The object of this article is to give a summary of these contributions and indicate their scope; for fuller details and descriptions, the reader may refer to the original papers published from time to time, of which the more important are given at the end of this article.

In the rocks of the Niniyur group itself, more algae have since been noticed, including *Neomeris*, *Acicularia*[3], *Clypeina*[3,7] and *Piania*; along with these there is also the 'unexpected' occurrence of a primitive form like *Holosporella*. The coral-reef limestones (Upper Albian) at the base of the Trichinopoly

Cretaceous succession have also revealed a rich algal flora, indicating that the algae played quite an important part in the building up of these reefs. Of outstanding significance is the occurrence in several rocks of this area, including the Niniyurs, of species of *Solenopora*[3], thus showing that the time-range of this genus extended up into the late Cretaceous, where it existed side by side with *Archaeolithothamnium*. Such an association of these two genera in the Cretaceous also provides valuable material for the study of the mutual chronological and evolutionary inter-relationships of the two important families of fossil algae which they represent. In the year 1937, J. Pia, S. R. N. Rao and K. S. Rao described fossil algae, including *Terquemella*, *Acicularia*, *Acetabularia* (?), and four species of *Neomeris* from the intertrappean beds near Rajahmundry[4]. This assemblage, which shows a distinct Tertiary aspect, is important not only in itself but also in throwing light on the age of the associated traps. A more recent examination of some of these intertrappeans which I have made shows that there are more of these Dasycladaceae here, probably including several other forms in addition to those mentioned above.

From the Lockhart limestone (Palæocene) of the Samana Range (now in Pakistan) S. R. N. Rao described in 1941 a rich algal flora belonging to the Melobesieae[5]. This includes several new species belonging to the genera *Archaeolithothamnium*, *Lithophyllum*, *Mesophyllum* and *Melobesia*. It may be noted that one of these species of *Archaeolithothamnium* (*A. samanensis*) found here shows "a concentric arrangement of the hypothallial rows"—a character which is considered as a distinctive feature of the genus *Lithophyllum*. Associated with these algae here, the solitary occurrence of a *Neomeris* (*Larvaria* ?) has also been noticed. From a limestone in Sind (Pakistan) probably also of Palæocene age, S. R. N. Rao and K. P. Vimal have quite recently (1955) described certain species of *Acicularia* and *Indopolia* representing the Dasycladaceae, together with the form *Boueina* belonging to the Codiaceae.

The occurrence of an abundant algal flora including both the Corallinaceae and Dasycladaceae in some of the limestones of doubtful Cretaceous or Eocene age of Assam was noticed in the year 1940; the Corallinaceae in this material have been studied in detail by K. S. Rao and the results embodied in a paper which he published in 1943[6]. Both the sub-families Melobesieae and Corallinae are represented here; of the former, which are by far the more abundant, he has described five species of *Archaeolithothamnium*, five of *Lithothamnium*, and one each of *Mesophyllum*, *Melobesia* and *Distichoplax*; and of the latter there is a new species of *Corallina*. The entire assemblage clearly indicates that the containing rocks are of early Tertiary age—a conclusion also supported by recent stratigraphical studies. The Dasycladaceae found in this material are yet to be described. These Assam limestones evidently constitute a rich storehouse of a varied and well-preserved algal flora, worthy of further attention.

Another important area where numerous fossil algae have been recently noticed is the Salt Range. The first report of this occurrence in the Eocene beds of this area (now in Pakistan) was made by me and K. S. Rao in 1939, recording the presence of *Dissocladella*, *Acicularia* and *Neomeris*, together with some surviving primitive forms like *Oligoporella* and *Diplopora*. More recently (1953) a rich algal flora from the lower Eocene limestones (of the Nammal gorge) of this region has been described by C. P. Varma[8]. He has recognized here some new species—three of *Archaeolithothamnium*, two of *Mesophyllum* and one of *Lithophyllum*; in this connexion he has also contributed a valuable discussion on the affinities of these and allied genera, especially in regard to *Mesophyllum*, where we can observe "a complete gradation from the *Lithothamnium* type to the *Lithophyllum* type of tissue among the various species of *Mesophyllum*". The same material also contains the remains of an alga very similar to the interesting form *Solenomeris*. Attention may also be directed to the three species of *Gymnocodium* recently described (1953) from the Permian beds of the same area.

The following classified list gives an idea of the variety and abundance of fossil algae described so far from the above areas: Dasycladaceae: *Holosporella* (2), *Triploporella* (1), *Oligoporella*, *Diplopora*, *Dissocladella* (1), *Indopolia* (2), *Acicularia* (4), *Orioporella* (1), *Neomeris* (9), *Clypeina* (3), *Terquemella* (1), *Acetabularia* (?) and *Piania* (1); Corallinaceae: (Melobesieae) *Archaeolithothamnium* (14), *Mesophyllum* (5), *Lithophyllum* (2), *Lithothamnium* (6), *Melobesia* (2), *Solenomeris* (1) and *Distichoplax*; (Corallinae) *Corallina* (1); Solenoporaceae: *Parachaetetes* (1) and *Solenopora* (3); Chaetophoraceae: *Palaeachlya*; Codiaceae: *Gymnocodium* (1) and *Boueina*. (The figures within brackets indicate the number of new species recognized.)

From the above brief review of the results of work done in India since the occurrence of abundant algae was first reported in 1931, it becomes clear that these algal remains "offer a vast field of research of which only the fringe has yet been touched". Apart from the Cretaceous and Eocene rocks which have yielded most of the forms mentioned above and will no doubt reveal many more on further examination in due course, we have a whole series of marine sedimentary rocks in India of earlier ages which are still 'untouched' from the point of view of their fossil algae. The farther back we go in time in this sedimentary succession, the more interesting will be their algal contents; and when we go down to the Precambrian rocks, of which we have such a good development in India, the search for fossil algae becomes positively exciting. A beginning in the study of the Precambrian algae in India has just been made by the record of a few remains by R. C. Misra in the rocks of the Vindhyan system, and of some algal (?) structures in the even older Cuddapah formations by M. R. S. Rao. The scope and opportunities for further explorations in India in the study of Precambrian algae are practically unlimited.

It is thus evident that a most promising field dealing with the study of fossil algae from rocks of various ages in India is still awaiting attention. It is earnestly hoped that this fruitful and fascinating line of research will be taken up and pursued in earnest before long; the results of these studies will undoubtedly be of the greatest value alike to the stratigraphical geologist and to the palæobotanist.

[1] Rao, L. R., *Proc. Ind. Acad. Sci.*, B, **44**, No. 4 (1956).
[2] Rao, L. R., and Pia, J., *Mem. Geol. Survey India, Pal. Indica*, **21**, 4 (1936).
[3] Rao, L. R., and Gowda, S. S., *Curr. Sci.*, **22**, 11 (1953); **23**, 6 (1954).
[4] Pia, J., Rao, S. R. N., and Rao, K. S., *Sitzungsber. Akad. Wiss. Wien*, **146**, 5 (1937).
[5] Rao, S. R. N., *Mys. Uni. J.*, **2**, 7 (1941); *J. Ind. Bot. Soc.*, M.O.P. Com. Vol. (1947).
[6] Rao, K. S., *Proc. Nat. Acad. Sci.*, **13**, 5 (1943).
[7] Varma, C. P., *Palaeobotanist*, Birbal Sahni Mem. Vol. (1952).
[8] Varma, C. P., *Proc. Nat. Acad. Sci. India*, **18**, 4 (1952); **19**, 3 (1953); **20**, 3 (1954).

LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications.

Presence of Triiodothyronine in Fowl

The observation of an unknown iodinated compound as a regular constituent of plasma and thyroid tissue of mice and rats[1-3] led to the discovery of 3-5 : 3′-triiodo-L-thyronine[4-7]. It has been established that triiodothyronine possesses all the thyroid hormone qualities of thyroxine when tested in a large number of species[8] and is more potent than thyroxine when tested by a variety of methods *in vivo* in mammals and amphibians[9]. Thus triiodothyronine has come to be recognized as being of physiological importance in laboratory mammals and man. However, little is known of the distribution of triiodothyronine in other classes of vertebrates, beyond the fact that it has been found in reptiles[10]. It seemed of interest to ascertain whether birds have the capacity to synthesize this compound.

Light Sussex cockerels, 100 days old, were given 250 μc. of iodine-131 by the subcutaneous route. 24 hr. later they were killed while under light ether anæsthesia, and the thyroid glands removed, minced and placed in ammonium-chloride ammonia : urea buffer (*p*H 8·6). Trypsin (1 mgm.) was added, and the glands were allowed to incubate for 24 hr. at 35° C. with a further addition of trypsin after 12 hr. After acidification with hydrochloric acid, the hydrolysate (*p*H 2·6) was extracted three times with butanol and the combined butanol extracts reduced to dryness under diminished pressure. The residue was taken up in methanol : ammonia (3 : 1) and applied to Whatman No. 1 filter paper for ascending chromatography in *n*-butanol–acetic acid–water (78 : 10 : 12 v/v) or *n*-butanol–dioxane (80 : 20 v/v) saturated with 2 *N* ammonium hydroxide systems. Thyroxine, triiodothyronine, diiodotyrosine, monoiodotyrosine and iodide were added as carriers. The positions of the carriers were visualized in ultra-violet light, and the triiodothyronine and/or thyroxine areas of the paper cut out, eluted with methanol–ammonia and re-chromatographed in a single dimension in *n*-butanol–dioxane–ammonia or in two dimensions in both solvent systems together with the appropriate carriers. The radioactivity of the paper was localized by radioautography and/or scanning with an automatic recording β-counter. The carriers were localized with diazotized sulphanilic acid and palladium chloride.

The results of two such experiments are shown in Fig. 1, and in both cases radioactivity was noted in the area of triiodothyronine. Other experiments, carried out on both males and females, from a cross between Light Sussex and Rhode Island Reds, in some cases following stimulation of the thyroid gland with thyroid-stimulating hormone, have confirmed these observations, but in no case was the percentage of radioactivity ascribed to triiodothyronine higher than 1 per cent of the total radioactivity extractable with butanol from the gland, or more than 5 per cent of the thyroxine radioactivity.

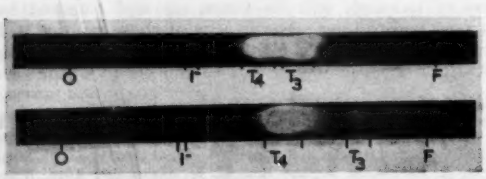


Fig. 1. Autoradiochromatograms of hydrolysed, butanol-extracted bird thyroid glands rechromatographed in butanol–dioxane–ammonia system. Bottom: elution of T_4–T_3 spot from a butanol–acetic acid–water chromatogram. Top: elution of T_3 spot from a butanol–dioxane–ammonia chromatogram. *O*, origin; I^- iodide; T_4 thyroxine; T_3, 3 : 5 : 3′-triiodothyronine

It may be concluded from the present experiments that the thyroid gland of the bird possesses the capacity to synthesize triiodothyronine, and that triiodothyronine presumably has a role in thyroid physiology in this class of vertebrates. This finding may take on added interest in the light of two curious findings, that birds have a low plasma protein-bound iodine value[11] and that triiodothyronine is no more potent than thyroxine in various biological tests done in birds[12,13].

Claire J. Shellabarger*
Rosalind Pitt-Rivers

National Institute for Medical Research,
The Ridgeway,
Mill Hill,
London, N.W.7.
Jan. 16.

* Special Fellow, National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland.

[1] Gross, J., and Leblond, C. P., *Proc. Soc. Exp. Biol., N.Y.*, **76**, 686 (1951).
[2] Gross, J., Leblond, C. P., Franklin, A. E., and Quastel, J. H., *Science*, **111**, 605 (1950).
[3] Gross, J., and Leblond, C. P., *Endocrinol.*, **48**, 714 (1951).
[4] Gross, J., and Pitt-Rivers, R., *Lancet*, **1**, 439 (1952).
[5] Gross, J., and Pitt-Rivers, R., *Biochem. J.*, **53**, 645 (1953).
[6] Roche, J., Lissitzky, S., and Michel, R., *C.R. Acad. Sci., Paris*, **234**, 997 (1953).
[7] Roche, J., Lissitzky, S., and Michel, R., *Biochim. Biophys. Acta*, **11**, 220 (1953).
[8] Barker, S. B., "Ann. Rev. Physiol.", **17**, 417 (1955).
[9] Selenkow, H. A., and Asper, jun., S. P., *Physiol. Rev.*, **35**, 426 (1955).
[10] Shellabarger, C. J., Gorbman, A., Schatzlein, F. C., and McGill, D., *Endocrinol.*, **59**, 331 (1956).
[11] Mellen, W. J., and Hardy, jun., L. B., *Endocrinol.*, **60**, 547 (1957).
[12] Shellabarger, C. J., *Poultry Sci.*, **34**, 1437 (1955).
[13] Newcomer, W. S., *Amer. J. Physiol.*, **190**, 413 (1957).

Enzymes of the Tricarboxylic Acid Cycle and Cytochrome Oxidase in the Fat Body of the Desert Locust

Various workers have shown the presence of some or all of the enzymes of the tricarboxylic acid cycle in insect muscle sarcosomes (for example, in *Musca domestica*[1], *Phormia regina*[2], *Apis mellifera*[3]) or in particulate fractions prepared from whole insects (for example, from *Aedes aegypti*[4]); but other specific insect tissues have received little attention. The insect fat body is often one of the most conspicuous organs and is known to carry out many reactions involving nitrogen metabolism[5]; but almost nothing has been recorded of other oxidative capabilities. As the biochemical function of the fat body is probably not primarily concerned with energy production (unlike muscle sarcosomes), it appeared of interest to examine this tissue.

The desert locust, *Schistocerca gregaria* Forsk., was used as a source of the fat body tissue which was carefully dissected out and gently homogenized in a Potter–Elvehjem blender. Fifth instar nymphs were used for most of the work. The presence of a number of enzymes associated with the tricarboxylic acid cycle was demonstrated in this tissue for the first

time. Using spectrophotometric assay methods, the presence was shown of a specific triphosphopyridine nucleotide-linked *iso*citric dehydrogenase, aconitase and fumarase. A diphosphopyridine nucleotide-linked malic dehydrogenase was demonstrated both by optical observation of the reduction of oxidized cytochrome c in the presence of cyanide, and manometrically by measurement of oxygen uptake in a Warburg apparatus. Cytochrome oxidase was present in the fat body, and was completely inhibited by $7{\cdot}5 \times 10^{-5}$ *M* cyanide and 94 per cent inhibited by 1×10^{-3} *M* azide. The cytochrome oxidase activity of the fat body showed a striking variation with age in the fifth stadium. It fell from a relatively high value at the beginning to about one-thirtieth towards the end of the stadium (that is, about 160 hr. after the fourth moult), and then rose sharply at the time of the fifth and final moult, and was at a high level in the young adult.

All efforts to demonstrate the presence of the condensing enzyme, succinic dehydrogenase and α-ketoglutarate oxidase were unsuccessful, both by optical and manometric methods. The apparent absence (or presence at a very low level below the sensitivity of the assay methods) of these key enzymes of the tricarboxylic acid cycle (although other enzymes which can take part in the operation of the cycle could readily be assayed), suggests that the cycle may not play an important part in the intermediary metabolism of the fat body of the locust.

We are indebted to the Anti-Locust Research Centre for financial support and encouragement in this work.

D. A. H. Hearfield
B. A. Kilby

Department of Biochemistry,
University, 9 Hyde Terrace,
Leeds 2.

[1] Sacktor, B., *Arch. Biochem. Biophys.*, **45**, 349 (1953).
[2] Lewis, S. E., and Slater, E. C., *Biochem. J.*, **58**, 207 (1954).
[3] Hoskins, D. D., Cheldelin, V. H., and Newburgh, R. V., *J. Gen. Physiol.*, **39**, 705 (1956).
[4] Gonda, O., Traub, A., and Avi-dor, Y., *Biochem. J.*, **67**, 487 (1957).
[5] Kilby, B. A., and Neville, E., *J. Exp. Biol.*, **34**, 276 (1957).

Phosphorylase Activity of Primary Rat Liver Cancer

The level of glycogen in the majority of tumours is considerably lower than in the normal homologous tissues[1]. According to Dickens and Weil-Malherbe[2] the glycogen content of primary rat hepatoma is lower than that of normal rat liver. As found recently by Goranson *et al.*[3], the phosphorylase activity of a transplantable rat hepatoma is very low. According to these authors, the addition of adenosine 5-phosphate greatly increases the phosphorylase activity of hepatoma tissue, up to the values found with normal rat liver. Hence the low content of glycogen in hepatoma might be due to a lack of adenosine 5-phosphate, rather than a deficiency in the amount of the phosphorylase itself. On the other hand, Le Page[4] found that rat hepatoma contains approximately twice as much adenosine 5-phosphate as normal rat liver. We have therefore studied the phosphorylase activity of primary rat liver carcinoma and the activating effect of adenosine 5-phosphate on it.

Discrete rat liver tumour nodes were selected for our experiments. The tumours were induced by feeding rats for 5 months with a semi-synthetic diet containing 0·6 per cent 4-dimethylaminoazobenzene[4]. Experimental and control animals were fasted for 24 hr. before killing; the tumour nodes were separated from the adjacent parenchyma, necrotic areas being carefully removed, and were homogenized at 0° with isotonic potassium chloride in a Potter–Elvehjem glass homogenizer. As it was found that the degree of homogenization is of importance for the phosphorylase activity, all experiments were made with homogenizers having an optimal clearance according to Potter[6].

Phosphorylase activity was determined by Sutherland's method[7], slightly modified according to the conditions of our experiments: the reaction was started by the addition of 0·5 ml. enzyme mixture to 1·0 ml. substrate solution (*p*H 6·1, glass electrode) containing glucose-1-phosphate (0·05 *M*), glycogen (5·7 mgm./ml.) and sodium fluoride (0·05 *M*). The enzyme mixture had the following composition: 0·5 ml. 2 per cent homogenate, 0·3 ml. *M* sodium fluoride, 0·2 ml. distilled water. When the activating effect of adenosine 5-phosphate was to be studied, 0·2 ml. of a solution of adenosine 5-phosphate (Pabst) was added, instead of water (final, 0·04 *M*). Test-tubes were incubated at 30°. At 0, 10 and 20 min., samples were taken, deproteinized with an aliquot of 5 per cent trichloracetic acid, and centrifuged. Inorganic phosphorus was determined in the supernatant by the method of Fiske–SubbaRow as adapted to a photoelectric colorimeter.

The phosphorylase activity of homogenates from normal rat liver (5 animals) and from primary rat liver cancer (7 animals) was studied. The effect of adenosine 5-phosphate (0·04 *M*), adenosine (0·04 *M*) and guanosine (0·02 *M*) was also investigated. In all experiments only fresh homogenates were used. The results of our experiments (Fig. 1) show that the phosphorylase activity of primary rat liver cancer is considerably lower (about one-third) than that of normal rat liver. The inorganic phosphorus released has the following average values: for tumour tissue,

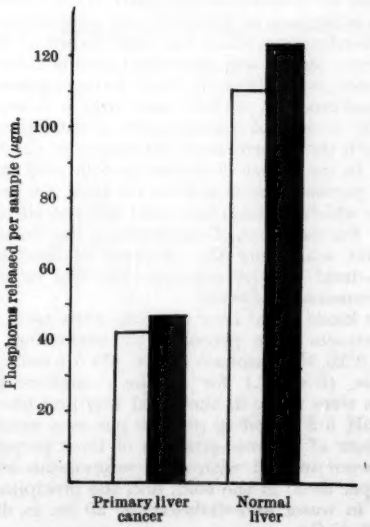


Fig. 1. Phosphorylase activity of normal rat liver and primary rat liver cancer. White, without adenosine 5-phosphate; black, with adenosine 5-phosphate

42·0 μgm. phosphorus, and for normal liver tissue, 109·8 μgm. phosphorus per sample in the conditions of our experiment. The adding of adenosine 5-phosphate in the above-mentioned concentrations increased the phosphorylase activity by 11 per cent for the primary rat liver carcinoma and by 11·2 per cent for normal rat liver. Adenosine and guanosine have no marked effect on phosphorylase activity.

The results of our experiments suggest that the low glycogen content in hepatic tumours could well be explained by the low phosphorylase activity of these tumours. Contrary to the data of Goranson *et al.*[2], our experiments indicate that rat liver tumours are characterized by a real decrease in the enzyme protein and not by a lack of adenosine 5-phosphate, the phosphorylase in tumour and normal liver homogenates being activated to the same degree by adenosine 5-phosphate.

A. A. Hadjiolov
K. I. Dancheva

Department of Biochemistry,
High Medical Institute,
Sofia.

[1] Butenand, A., and Dannenberg, H., "Die Biochemie der Geschwülste in Handbuch der allgemeine Pathologie", 6, Part 3, 107 (Springer, 1956).
[2] Dickens, F., and Weil-Malherbe, H., *Cancer Res.*, 3, 73 (1943).
[3] Goranson, E., McBride, J., and Weber, G., *Cancer Res.*, 14, 227 (1954).
[4] Hadjiolov, A. A., *Bull. Inst. Morphol. Acad. Sci. Bulgarie*, 2, 423 (1957).
[5] Le Page, G. A., *Cancer Res.*, 8, 193 (1948).
[6] Potter, V. R., in Colowick, S., and Kaplan, N., "Methods in Enzymology", 1, 10 (Acad. Press, New York, 1955).
[7] Sutherland, E. W., in Colowick, S., and Kaplan, N., "Methods in Enzymology", 1, 213 (Acad. Press, New York, 1955).

Occurrence of Independent Systems for the Cleavage of Folic Acid and Citrovorum Factor in Rat Liver

Recent work has demonstrated that extracts from animal tissues can actively degrade folic acid[1,2] and citrovorum factor[3] at the C_9—N_{10} linkage with the liberation of a diazotizable amine. Whether the same enzyme systems are involved in the cleavage of the two substrates is, however, not established. The horse-liver fraction which has been shown to degrade citrovorum factor was inactive towards folic acid[3]. In a recent paper, Dinning *et al.*[4] have suggested that the transformation of folic acid into a diazotizable amine by slices and homogenates of rat livers takes place with the intermediate formation of citrovorum factor. In the course of studies on folic acid degradation by purified fractions from rat liver, we prepared extracts which degrade folic acid but not citrovorum factor. Furthermore, *A*-methopterin has been found to inhibit selectively the cleavage of folid acid in acetone-dried powder extracts, but the citrovorum factor remains unaffected.

Three kinds of rat liver extracts were used. Fresh liver extracts were prepared by extraction of the liver in 0·15 *M* phosphate buffer, *p*H 5·5 and dialysis in water (0–4° C.) for 20 hr.; acetone–powder extracts were made in the usual way and phosphate buffer *p*H 5·5 added to give 10 per cent extracts.

Extracts of acetone–powders of liver prepared as above were treated with solid ammonium sulphate (30–70 per cent) in the cold, and the precipitate suspended in water and dialysed for 20 hr. in distilled water (0–4° C.).

Enzyme activity was determined by following the liberation of the aromatic amine, according to Bratton and Marshall[5]. Non-substrate blanks were determined by incubating tubes with all constituents of the reaction mixture except folic acid. The latter was added at the end of the incubation period.

Table 1. Co-factor Requirements

The complete system consisted of 150 μgm. of folic acid or citrovorum factor, 0·06 *M* phosphate buffer, $1·5 \times 10^{-3}$ *M* adenosine triphosphate, $1·0 \times 10^{-2}$ *M* manganese chloride, $1·0 \times 10^{-2}$ *M* sodium citrate, $1·0 \times 10^{-4}$ *M* coenzyme I, 1 ml. acetone powder extract. Total volume = 2 ml.; time of incubation, 3 hr.; temperature of incubation, 37° C. (The values given have been corrected for the non-substrate blank)

Sample No.	System	Folic acid degraded at *p*H 5·5 (μgm.)	Citrovorum factor degraded at *p*H 6 (μgm.)
1	Complete system	110·70	36·50
2	Adenosine triphosphate omitted	73·30	8·85
3	Manganese chloride omitted	75·30	17·30
4	Sodium citrate omitted	12·70	12·90
5	Coenzyme I omitted	8·50	9·90
6	All the above omitted	nil	5·5

Table 2. Effect of Some Substances on Enzymes degrading Folic Acid and Citrovorum Factor

Complete system as in Table 1. Total volume = 2 ml.; time of incubation, 3 hr.; temperature of incubation, 37° C. (The values given have been corrected for the non-substrate blank.)

Sample No.	Supplements added to the complete system	Folic acid degraded at *p*H 5·5 (μgm.)	Citrovorum factor degraded at *p*H 6 (μgm.)
1	Nil	92·7	35·7
2	*A*-methopterin (10 μgm.)	2·0	35·0
3	L-Glutamic acid (10^{-3} mole)	96·3	35·0
4	N-Formylglutamic acid* (10^{-3} mole)	80·3	31·2

* N-Formylglutamic acid was a gift from Dr. H. R. V. Arnstein.

Table 3. Comparative Degradation of Folic Acid and Citrovorum Factor by Different Rat Liver Preparations

The incubation mixture consisted of 150 μgm. folic acid or citrovorum factor, same co-factors as in Table 1, and enzyme extract as specified. Buffer, 0·06 *M* phosphate, *p*H 6; time of incubation, 3 hr.; temperature of incubation, 37° C. (The results have been corrected for the non-substrate blank)

Source of enzyme	Folic acid degraded (μgm.)	Citrovorum factor degraded (μgm.)	Ratio of citrovorum factor degraded to folic acid
Dialysed fresh liver extract (1 ml.)	54·3	12·7	0·233
Acetone-powder extract (1 ml.)	79·3	35·4	0·446
30–70 per cent ammonium sulphate fraction from acetone powder extract (0·5 ml.)	87·7	8·5	0·097

Dialysed extracts of acetone-dried powders from rat livers catalyse the liberation of a diazotizable amine from folic acid and citrovorum factor. The activity in presence of either substrate is dependent on coenzyme I, adenosine triphosphate, manganese ions, and citrate. These co-factors have also been found to be necessary for the cleavage of folic acid by chicken liver extracts[3]. Results of experiments given in Table 1 show that omission of any one of the supplements causes a considerable reduction in activity in presence of either substrate. In a publication, which has reached us while this communication was in preparation, Silverman *et al.*[6] report the purification of an enzyme system from hog liver which cleaves citrovorum factor. The latter enzyme differs in some of its properties from the rat liver preparations described here. It is activated by

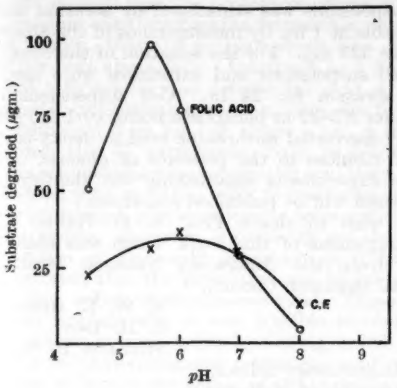


Fig. 1

L-glutamic acid, inhibited by N-formylglutamic acid and apparently does not need any of the above co-factors required by the rat liver system. Results in Table 2 show that L-glutamic acid and N-formylglutamic acid produce negligible effects on the system cleaving folic acid or citrovorum factor in rat liver. *A*-methopterin selectively inhibits the folic acid degradation but citrovorum factor is still unaffected. Furthermore, the folic acid degrading system can be largely separated from the activity towards citrovorum factor by ammonium sulphate fractionation of extracts made from acetone–dried powder (Table 3). The relation of *p*H to activity in presence of the two substrates is illustrated in Fig. 1. The two curves show marked differences. The *p*H optimum for folic acid is at *p*H 5·5 and for citrovorum factor at *p*H 6. Above *p*H 6 the activity in presence of folic acid falls rapidly. This may be a possible explanation for the higher rate of degradation of citrovorum factor obtained by Dinning *et al.*[4] in rat liver homogenates, for their experiments were carried out at *p*H 7·4.

The findings reported here indicate that, in rat liver, separate enzyme systems catalyse the conversion of folic acid and citrovorum factor into a diazotizable amine. The present results do not show whether these conversions proceed through the formation of tetrahydrofolic acid, as has been demonstrated for the system reacting with citrovorum factor in hog liver[6]. Differences between the co-factor requirements and the lack of effect produced by L-glutamic acid indicate, however, that the enzymes catalysing the cleavage of citrovorum factor in the two animal species may not be identical.

We thank Dr. V. R. Khanolkar, director of this Centre, for the facilities made available to us during the course of this work, and the authorities of the Lady Tata Memorial Trust for a scholarship awarded to one of us (U. W. K.).

U. W. Kenkare
Beatriz M. Braganca
Enzyme Chemistry Department,
Indian Cancer Research Centre,
Bombay.

[1] Braganca, B. M., Aravindakshan, I., and Ghanekar, D. S., *Biochim. Biophys. Acta*, **25**, 623 (1957).
[2] Futtermann, S., and Silverman, M., *J. Biol. Chem.*, **224**, 31 (1957).
[3] Keresztesy, J. C., and Silverman, M., *J. Amer. Chem. Soc.*, **75**, 1512 (1953).
[4] Dinning, J. S., Sime, J. T., Work, P. S., Allen, B., and Day, P. L., *Arch. Biochem. Biophys.*, **66**, 114 (1957).
[5] Bratton, A. C., and Marshall, E. K., *J. Biol. Chem.*, **128**, 537 (1939).
[6] Silverman, M., Keresztesy, J. C., Koval, G. J., and Gardiner, R. C., *J. Biol. Chem.*, **226**, 83 (1957).

A Possible Intermediate in the Biosynthesis of Tryptophan : I-Deoxy-l-N-o-Carboxyphenyl-Ribulose

During an investigation of compounds formed by washed cell suspensions of mutant strains of *Aerobacter aerogenes* blocked at various stages in tryptophan synthesis, two strains (*N*3–33 and *N*2–18) were found to form a compound which showed an intense blue-violet fluorescence under ultra-violet light, but the absorption spectrum of which did not correspond to any known compound. Neither strain of *A. aerogenes* would grow in the minimal medium described by Davis and Mingioli[1] unless either tryptophan or indole was added and they were therefore presumably unable to convert anthranilic acid to indole.

Cell suspensions were incubated with a mixture of glucose (0·1 *M*), ammonium chloride (0·005 *M*), magnesium sulphate (10^{-4} *M*) and phosphate buffer (0·1 *M*, *p*H 7·7) as described by Gibson, Jones and Teltscher[2]. After removal of the cells, spectrophotometric examination of the supernatant fluid showed the presence of a compound with a spectrum similar in some respects to that of anthranilic acid (Fig. 1). The compound was readily extracted by ethyl acetate after the supernatants had been acidified, but concentration of these extracts to dryness resulted

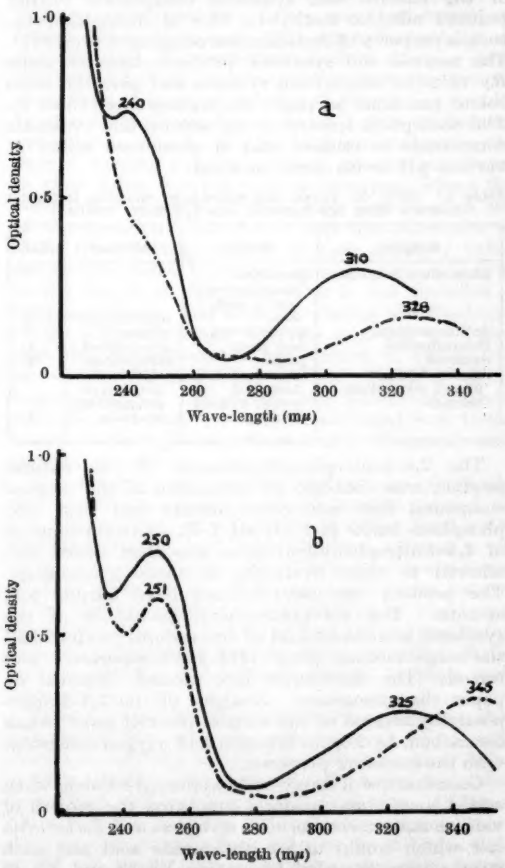
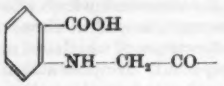
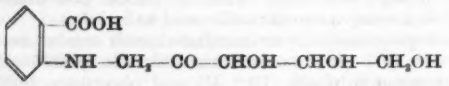


Fig. 1. Ultra-violet absorption spectra of: (*a*) anthranilic acid (10^{-4} *M*) 0·1 *M* phosphate; (*b*) experimental sample with 0·1 *M* phosphate as blank. ———, Spectrum at *p*H 7·7; –·–·–, spectrum at *p*H ~ 2

in decomposition of the compound. It has not been found possible to isolate the pure compound, but its identity has been established by the absorption spectrum, paper chromatography and by the formation of the crystalline 2,4-dinitrophenylhydrazone. The examination of the absorption spectra of a number of anthranilic acid derivatives indicated that the spectrum was consistent with the structure



The compound appears to be 1-deoxy-1-N-*o*-carboxyphenyl-ribulose.



This compound was suggested[2] as a possible intermediate in the conversion of anthranilic acid to indole and is the Amadori rearrangement product of the riboside or arabinoside of anthranilic acid. It has been prepared from both the D-riboside and D-arabinoside and although not yet isolated was readily detected on paper chromatograms by reagents such as 2,3,5-triphenyltetrazolium chloride. Concentrates of the natural and synthetic compounds readily reduced alkaline methylene blue at room temperature, a property of Amadori rearrangement products[4]. The natural and synthetic products had the same R_F value in two solvent systems and gave the same colour reactions on paper chromatograms (Table 1). The absorption spectra of the natural and synthetic compounds in ethanol and in phosphate buffer at various *p*H levels were identical.

Table 1. Tests on Paper Chromatograms showing Identical Results with the Natural and Synthetic Product

Reagent	Result	Inference	Ref.
Ammonium molybdate	green-blue changing rapidly to dark blue	sugar present	6
Aniline-acetate	purple-brown	pentose	7
Phloroglucinol	dark green	keto-pentose	8
Anthrone	purple	keto-pentose	9
Alkaline 2,3,5,-triphenyl tetrazolium chloride	immediate intense red colour without heating	evidence for Amadori rearrangement products	10 4

The 2,4-dinitrophenylhydrazone of the natural product was obtained by extraction of the original compound first into ethyl acetate and then into phosphate buffer (0·1 *M*, *p*H 7·7). An acid solution of 2,4-dinitrophenylhydrazine was then added and allowed to react overnight at room temperature. The product was recrystallized from alcohol and acetone. The 2,4-dinitrophenylhydrazones of the synthetic compound and of the natural product had the same melting point (213–215°) separately and mixed. The derivatives also seemed identical on paper chromatograms. Analysis of the 2,4-dinitrophenylhydrazone of the natural product gave values for carbon, hydrogen, nitrogen and oxygen consistent with the structure proposed.

Concentrates of deoxy-carboxyphenyl-ribulose when added to minimal medium supported the growth of various mutant strains of *A. aerogenes* and *Escherichia coli* which would utilize anthranilic acid and such strains were cross-fed by strains *N*3–33 and *N*2–18 when tested by the technique described by Gibson and Jones[5]. The formation of the compound by cell suspensions was stimulated by aeration and was detectable at 1 hr. by measurement of the absorption peak at 325 mμ. For the isolation of the compound, the cell suspensions and substrates were incubated with aeration for 24 hr. Cell suspensions of *A. aerogenes* *N*3–33 in phosphate buffer (0·1 *M*, *p*H 7·7) readily converted anthranilic acid to deoxy-carboxyphenyl-ribulose in the presence of glucose. Details of the experiments establishing the identity of the compound will be published elsewhere.

We wish to thank Prof. S. D. Rubbo for his encouragement of this work which was aided by a grant from the Australian National Health and Medical Research Council.

F. W. E. Gibson
C. H. Doy
Shelagh B. Segall

Bacteriology School,
University of Melbourne.
Oct. 22.

[1] Davis, B. D., and Mingioli, E. S., *J. Bact.*, **60**, 17 (1950).
[2] Gibson, F., Jones, M. J., and Teltscher, H., *Biochem. J.*, **64**, 132 (1956).
[3] Yanofsky, C., *J. Biol. Chem.*, **223**, 171 (1956).
[4] Hodge, J. E., "Adv. Carbohydrate Chem.", **10**, 169 (1955).
[5] Gibson, F., and Jones, M. J., *Aust. J. Sci.*, **17**, 33 (1954).
[6] Aronoff, S., and Vernon, L., *Arch. Biochem.*, **28**, 424 (1950).
[7] Aronoff, S., "Techniques of Radiobiochemistry" (Iowa State College Press, Ames, 1956).
[8] Borenfreund, E., and Dische, Z., *Arch. Biochem. Biophys.*, **67**, 239 (1957).
[9] Johanson, R., *Nature*, **172**, 956 (1953).
[10] Trevelyan, W. E., Procter, D. P., and Harrison, J. S., *Nature*, **166**, 444 (1950).

Mode of Action of Chelates in protecting against *Clostridium perfringens* Toxin

It was reported previously that ethylenediamine tetraacetic acid was capable of protecting mice against the toxin of *Clostridium perfringens*[1]. The protection thus afforded was shown to be probably due to the removal of a metallic ion or ions necessary for the activity of the toxin from the area where the toxin was acting. Inasmuch as the metallic ion or ions necessary for the action of the toxin apparently were higher in the preferential chelation series than calcium, the calcium chelate was shown to be able to protect when given systemically[2]. In this report, studies on the duration of the protection afforded by calcium ethylenediamine tetraacetic acid are presented and their implications are discussed.

The toxin, calcium ethylenediamine tetraacetic acid, and methods of administration of both of these were the same as described previously[2]. 2·5, 5 and 30 per cent solutions of calcium ethylenediamine tetraacetic acid were injected intradermally into mice; at various time-intervals thereafter the mice were injected at a different site with toxin and the deaths occurring within a 48-hr. period were recorded. The results of three typical experiments are presented in Table 1.

Table 1. Duration of Effect of Calcium Ethylenediamine Tetraacetic Acid in protecting Mice against *Cl. perfringens* Toxin

Calcium ethylenediamine tetraacetic acid injected (per cent)	Time (hr.) after injection of calcium ethylenediamine tetraacetic acid that toxin was injected. Groups of ten mice were used for each time-interval and the number that died is recorded									
	0	½	1	2	3	4	6	9	12	15
2·5	0	5	9	10	—	—	—	—	—	—
5	0	—	—	5	—	10	10	—	—	—
30	0	—	—	—	0	—	3	6	9	10

It can be seen that the protective effects of 2·5 per cent calcium ethylenediamine tetraacetic acid lasted about 30 min., that of 5 per cent about 2 hr., and that of 30 per cent about 9 hr. Inasmuch as 2·5 per cent calcium ethylenediamine tetraacetic acid is capable of completely protecting mice against the toxin and this protection lasts for but about ½ hr., it indicates that once the *Cl. perfringens* toxin is inhibited by calcium ethylenediamine tetraacetic acid there is some mechanism present in the body to inactivate it. This ability of calcium ethylenediamine tetraacetic acid to protect against the toxin is certainly not due to the inactivation of the toxin inasmuch as it has been demonstrated that the protection can be reversed by either zinc, cobalt or manganese ions[1]. These results also may explain why it was possible to render the toxin inactive in mice simply by mixing it with a small quantity of ethylenediamine tetraacetic acid[1]. If it is necessary to inhibit the action of the toxin for a short interval of time to render it non-toxic in the body, the period of time that the ethylenediamine tetraacetic acid remained at the injection site was sufficient to allow the inactivating mechanism in the body to come into play.

It was previously suggested that calcium ethylenediamine tetraacetic acid protected against the toxin when given systemically by binding the 'free' trace metals that were available in the body[2]. If this interpretation obtains, the data presented here would indicate that, although the level of 'free' trace metals is reduced to the point where the toxin is inactive with 2·5 per cent ethylenediamine tetraacetic acid, the trace metal equilibrium is restored within ½ hr. by the release of metals from depots in the body. The fact that higher concentrations of calcium ethylenediamine tetraacetic acid protect for proportionately longer periods of time suggests that sufficient calcium ethylenediamine tetraacetic acid remains in the circulation during the respective time-intervals to bind the metals that are released. An implication of these findings is that in attempts to use chelating compounds as detoxifying or therapeutic agents in other diseases a mode of administration other than that used with *Cl. perfringens* toxin will probably be necessary. In order to inhibit the growth of micro-organisms *in vivo* with agents such as antibiotics it is necessary to maintain the inhibitory level of the antibiotic until the micro-organisms are no longer active. In instances where the removal of 'free' trace metals may be inhibitory to the activities of a micro-organism *in vivo*, this condition probably would have to be maintained until the activities of the micro-organisms are suppressed to the point where they are no longer harmful to the body. A mode of administration of a chelating compound such that the level in the body is maintained sufficiently high to keep the 'free' trace metals at the requisite low level would be indicated in such instances.

In another series of experiments toxin was injected into groups of 10 mice, and at intervals of 2 hr. thereafter 5 per cent calcium ethylenediamine tetraacetic acid was injected at another site. The results of these experiments can be summarized as follows: If calcium ethylenediamine tetraacetic acid was given within 6 hr. after the injection of toxin, the mice survived; if it was given at 8 or 10 hr., the majority of mice, and all of them in some experiments, died. The median death time for the dose of toxin used was about 10 hr.; after 6 hr. all the mice were severely ill with a small percentage of deaths occurring by this time. It would appear that the action of the toxin is cumulative, and if its activity is inhibited by calcium ethylenediamine tetraacetic acid before it reaches the lethal level the mice are able to recover.

This investigation was supported by Grant *E*–1099 of the National Institutes of Health.

MERWIN MOSKOWITZ

Department of Biological Sciences,
Purdue University,
Lafayette, Indiana.

[1] Moskowitz, M., *et al.*, *Science*, **123**, 1077 (1956).
[2] Moskowitz, M., *Proc. Soc. Exp. Biol. Med.*, **92**, 706 (1956).

Adherence and Viscosity of Blood Contacting Foreign Surfaces, and the Plasmatic Zone in Blood Circulation

THE cell-free marginal zone of plasma in vessels of living animals, due to the axial accumulation of red blood cells, was already known to Haller and Spallanzani in the eighteenth century[1]. The formation of this zone was later studied by Poiseuille[1] in arteries, veins and capillaries in various species of the living animal. He found that, with increased velocity, the thickness of this layer was augmented. Recently, in the course of investigations on the flow of blood columns using a tilting viscometer (Scott Blair, G. W., personal communication), we made the following observation. If an index of human or rabbit blood, containing 0·01 mgm. heparin/ml. as anticoagulant, be permitted to flow down a large capillary tube held at varying angles from the vertical, then films of blood of varying thickness can usually be seen macroscopically on the wall after the column has passed. This finding led to investigations on the flow of varying indexes of blood, plasma and serum in glass tubes 0·5–1·4 mm. in diameter, where the Reynolds number is so small that turbulence is out of the question. We found that the adherence of fluids from moving indices in such capillary tubes can be determined by simply measuring the reduction in the length of the index after it has travelled a certain distance, and then calculating the thickness of the adherent layer. By this method, we found that this phenomenon is not limited to blood, but can also be detected when cell-free plasma or serum is used instead. Moreover, this adherence is not dependent on glass surfaces, as we found it in tubes coated with silicone, 'Lusteroid', 'Perspex' and paraffin as well.

The question now arises whether the plasmatic zone in the living blood vessels can be related to this phenomenon of adherence in tubes lined with various surfaces, and also whether adherence of fluids in capillary tubes can be found to be a general physical phenomenon. In examining how the nature of the surface affected the adherence of blood, we discovered that the velocity of flow differed markedly with all surfaces at various angles of tilt. With higher velocities, more blood adhered to glass and other surfaces.

Table 1 shows the effect of various surfaces on the velocity of flow and the adherence of blood and plasma in capillary tubes of radii ranging from 507μ to 492μ. Rabbit's heart blood and plasma from the same blood withdrawal was used in all tests. Indexes 100 mm. long were run in duplicate and triplicate along lengths ranging from 441 mm. to 426 mm. and at 20° tilt of the tube; the same glass tube being used throughout the entire series of experiments, comparative tests were run with coatings of

Table 1. Flow and Adherence Values of Indexes. 100 mm. Long, of Blood and Plasma of Same Rabbit Heart Blood (0·01 mgm. Heparin/ml.) Sample Contacting Various Surfaces at 20° C. and at 20° Angle of Tilt. Length of Run varying from 426 to 441 mm.

Surface	Calculated radius of tube (μ)	Fluid	Flow		Adherence	
			Time (sec.)	Velocity (mm./sec.)	Vol. of index adherent (per cent)	Calculated thickness of layer (μ)
Glass	507	Blood	54·0	8·1	9·5	5·6
		Plasma	25·4	16·8	1·8	1·1
'Perspex'	492	Blood	44·8	9·6	3·8	2·2
		Plasma	23·7	18·0	2·5	1·4
'Lusteroid'	498	Blood	68·7	6·2	2·8	1·6
		Plasma	43·1	9·9	1·3	0·7
Paraffin	498	Blood	410·6	1·1	16·3	9·2
		Plasma	193·0	2·3	9·0	5·2
Silicone	498	Blood	41·0	10·4	1·5	0·8
		Plasma	21·8	19·6	0·5	0·3

'Lusteroid', paraffin wax, 'Perspex' and silicone, after control runs against the uncoated glass surface. Table 1 shows clearly that both blood and plasma indexes from the same blood withdrawal have widely varying velocities of flow depending on the surfaces with which they are in contact. For any particular surface, plasma always flowed faster than blood. As can be seen, variations in diameter of the tube due to the coatings being of different thickness cannot be made responsible for these wide divergencies in the velocity of plasma and blood indexes from the same blood sample. Nor do the anomalous flow properties of blood entirely explain these phenomena, since plasma behaves like a Newtonian fluid[2]. Table 1 also demonstrates that the amount of adherence, rather than being directly related to the velocity of the index *per se*, depends on the nature of the surface as well, with silicone exhibiting least adherence and paraffin the most in spite of the very low velocity of flow with the latter. For each surface, plasma gave relatively lower values of adherence than those of blood. The above phenomena occurred with blood–air interfaces. However, similar findings were obtained in a system without air through capillary tubes, 600 mm. long, maintained horizontally. A column of oil was merely used to act as a piston without coming in contact with the blood over the measured region of the tube.

Our results appear to be significant because: (1) Measurements of the adherence of blood and plasma have not been made thus far. The fact that blood and plasma adhere to the silicone and paraffin suggests that the well-known physical anticoagulant effect of these surfaces may be independent of the phenomenon of adherence. (2) Observations on a marginal layer in living blood vessels as well as in capillary tubes have always been related to whole blood or to suspensions of red cells in different fluids[3,4] but have never been considered for plasma without suspended red cells. It appears from our observations that the immobile layer of plasma at the blood vessel wall, as established by Poiseuille[1], is not necessarily due to a suspension of red cells, but may as well exist when plasma alone flows through a living blood capillary, for example, in plasma skimming. (3) Viscosity studies of blood and plasma have been made in the past only in glass tubes. Hence, until the nature and physical properties of the surface lining the blood vessel[5] have been established, the widely divergent values we report for blood and plasma in contact with various surfaces make it seem doubtful whether the results of all previous studies on viscosity of shed blood and plasma in glass tubes can be applied to blood and plasma circulating in living blood vessels.

Nevertheless, our observations invite new rheological studies with blood and plasma from different subjects of various species in health and disease. These findings on adherence of blood and plasma could explain why arteries and veins may differ outside of their morphological structures, because, due to the various velocities in vessels of varying diameters, one should then expect various degrees of thickness of adherent layers. Finally, our observations may throw a new light on a number of problems related to the physiology of blood circulation as well as on certain vascular disorders associated with changes in the structure of the inner or endo-endothelial lining[5] facing the lumen of the vessel.

I am indebted to Dr. G. W. Scott Blair for his helpful discussions.

This work was aided in part by a grant from the National Science Foundation, Washington.

Alfred L. Copley

Medical Research Laboratories,
Charing Cross Hospital,
London, W.C.2. Nov. 22.

[1] Poiseuille, J. L. M., *Ann. Sci. Naturelles*, 2, Sér. Zoologie, **5**, 111 (1836); "Acad. Sci. Savants étrangers", **7**, 44 (Impr. Royale, Paris, 1839).

[2] Copley, A. L., Krchma, L. C., and Whitney, M. E., *J. Gen. Physiol.*, **26**, 49 (1942).

[3] Copley, A. L., Proc. Int. Congr. Rheology Scheveningen 1948, 1, 47 (North-Holland Pub., Amsterdam, 1949); *J. Colloid Sci.*, **7**, 323 (1952).

[4] Bayliss, L. E., "Deformation and Flow in Biological Systems", edit. by Frey-Wyssling, A., 355 (North-Holland Pub., Amsterdam, 1952).

[5] Copley, A. L., Abstr. Comm. 19, Int. Physiol. Congr., Montreal, 1953, 280; *Arch. Int. Pharmacodyn.*, **99**, 426 (1954); *Aerztl. Forsch.*, **11**, I/114 (1957); *Lancet* I, 102 (1957) and ii, 1119 (1957).

Isolation of Reserpine from *Vinca rosea* Linn.

In recent years considerable interest has been aroused in the use of the alkaloid reserpine in the treatment of hypertension and various mental disorders. Various plants in the family of Apocynaceae other than *Rauwolfia* have been investigated, and among them the isolation of reserpine from *Alstonia constricta*[1] is noteworthy. With the view of detecting the presence of reserpine in the plants of the Apocynaceae family we have studied the plant *Vinca rosea*. Several alkaloids have been reported from this plant: vinceine (melting point, 259–260° C.)[2], and vincaine (melting point, 250–252° C.)[3], which appears to be identical or stereoisomeric with δ-yohimbine. According to Beereboom *et al.*[4], vinceine and vincaine are indeed identical with ajmalicine, and the same authors have reported[4] the presence of serpentine (melting point, 156° C., with decomposition) and a new alkaloid, $C_{20-21}H_{26-28}N_2O_3$ (melting point, 200–201° C.) which they propose to name 'lochnerine'. Recently, ursolic acid (melting point, 280° C.)[5] has been reported present in *Vinca rosea*.

We have been able to isolate an alkaloid (melting point, 265–266° C.) from the ether-insoluble weakly basic resin of the roots of *Vinca rosea* grown in West Bengal. This is now confirmed to be reserpine by determination of the mixed melting point with authentic reserpine, as well as by the R_F values of the sample isolated, authentic reserpine and the mixture of the two. Whatman paper No. 1 and a

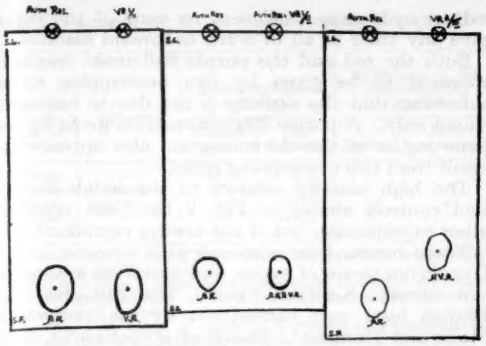


Fig. 1. *A.R.*, Authentic reserpine; *V.R.*, reserpine isolated from *Vinca rosea*; *N.V.A.*, a new alkaloid isolated from *Vinca rosea*

n-butanol/hydrochloric acid/water system were employed for chromatography.

Another alkaloidal base (melting point, 242° C., with decomposition) with R_F 0·70 (R_F reserpine 0·86) has been isolated from the same fraction. Further work on this alkaloid is in progress. Experimental details of the isolation and further work on this plant will be published elsewhere.

N. K. Basu
B. Sarkar

Department of Pharmaceutics,
Banaras Hindu University,
Banaras.

[1] Crow, W. D., and Gree, N. Y., *Austral. J. Chem.*, **8**, 461 (1955).
[2] Paris, R. A., and Moyse-Mignon, Helen, *C.R. Acad. Sci., Paris*, **236**, 1903 (1953).
[3] Chatterjee, A., *Science and Culture*, **20**, 368 (1955).
[4] Beereboom, J. J., Pakrashi, S. C., and Djerassi, C., *Chem. and Indust.*, 173 (1956).
[5] Chatterjee, A., and Roy, S., *J. Ind. Chem. Soc.*, **34**, 340 (1957).

Auxins in Immature Maize Kernels

Ether extracts of maize-kernel pulp at *p*H 6 contain only part of the growth-promoting activity of the kernels. The juice expressed from the ether-extracted kernels is still highly active in the straight-growth test. In Table 1 the activity of the expressed juice from ether-extracted maize kernels is compared with that of an equivalent concentration of ether-extractable material.

At first, it seemed possible that these results were due to incomplete extraction of indole-3-acetic acid. To test this, ether-extracted juice was partly purified by ethanol (4 vol.) precipitation of inactive material. After removal of ethanol, the juice was acidified to *p*H 3·6 with sulphuric acid, exhaustively extracted with ether and then neutralized with barium hydroxide solution.

The activity remaining in the acid/ether-extracted juice is compared with that of the acid/ether extract

Table 1. Effect of Maize Extracts on Wheat Coleoptiles

	Final length sections (mm.)		
	Original solution	× 10 dilution	× 100 dilution
Ether extractable fraction	11·9	19·4	14·1
Juice after ether extraction	11·2	19·2	16·5
	1 mgm./l.	0·1 mgm./l.	0·01 mgm./l.
Indole-3-acetic acid	19·2	16·5	14·1
Water controls	14·5		

Initial length of sections, 10 mm.

Table 2

	Final length of sections (mm.)		
	Original solution	× 10 dilution	× 100 dilution
Acid/ether extractable fraction	12·9	16·1	14·4
Juice after acid/ether extraction	16·2	18·7	15·8
	1 mgm./l.	0·1 mgm./l.	0·01 mgm./l.
Indole-3-acetic acid	17·8	15·8	14·3
Water controls	13·4		

Initial length of sections, 10 mm.
In this experiment, oat coleoptiles were used, as in the straight-growth test of Bentley and Housley (ref. 1).

in Table 2. In this experiment and the subsequent ones activity was tested by the *Avena* straight-growth test of Bentley and Housley[1].

Table 2 shows that approximately only a further 10 per cent of the activity of the ether-extracted juice can be obtained under these conditions. Indole-3-acetic acid would be completely removed by this treatment. The activity of the juice is due, therefore, to some constituent which is not indole-3-acetic acid. For an investigation of this unknown active constituent, the juice was first purified as before by ethanol precipitation. It was then evaporated to a syrup and exhaustively extracted with ether at *p*H 6.

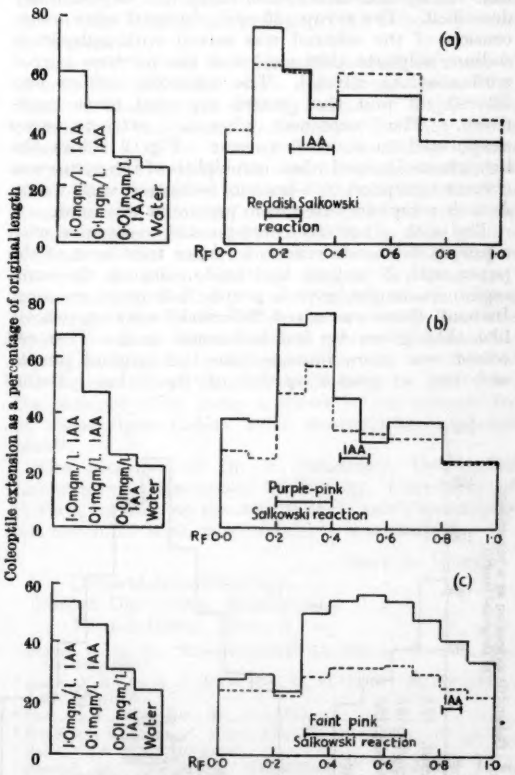


Fig. 1. Histograms of ether-extracted maize juice chromatographed in: (*a*) *n*-butanol saturated with *N* ammonium hydroxide solution; (*b*) *iso*propanol: water (4 : 1) containing a trace of ammonia; (*c*) acetone: water (1 : 1). Broken lines show activity extractable by ether after chromatography, while full lines show activity not extractable under these conditions. Assay was by the *Avena* coleoptile straight-growth test

The results in each histogram in Fig. 1 were obtained from material equivalent to 4 gm. of maize-kernel pulp. Fig. 1 shows that the activity of the juice appears at lower R_F values than does indole-3-acetic acid.

From strips of paper chromatograms run in the presence of ammonia some activity can be extracted by ether. The activity coincides with a Salkowski-reacting region on the paper. In the three chromatograms in Fig. 1, the colour of the Salkowski reaction was rather patchy and in the case of the paper run in acetone/water, very faint and indistinct. The other two papers, run in the presence of ammonia, appeared to show that a substance with a purple Salkowski reaction was present. At slightly greater R_F values, though not completely separated from the purple zone, there was a reddish Salkowski reaction.

The general impression obtained was that in ammoniacal solvents a substance with a purple Salkowski reaction was giving rise to a substance with a red Salkowski reaction and a greater R_F value. The indistinctness of the spots was thought to be due to the effect of large amounts of other constituents present.

A further concentration of the juice was made. Juice equivalent to about 200 gm. pulped maize kernels was partly purified by ethanol precipitation followed by exhaustive ether extraction as previously described. The syrup (25 ml.) obtained after evaporation of the ethanol was mixed with anhydrous sodium sulphate (100 gm.) and the mixture stirred with absolute ethanol. The ethanolic extract was filtered off and the process repeated three more times. The combined ethanolic extracts were evaporated to a small volume. Fig. 2 shows the histogram obtained when one-eighth of the syrup was chromatographed in *n*-butanol saturated with water. A tank completely free from ammonia was used.

The peak of activity corresponded to a region with a purple Salkowski reaction. After treatment of the paper with *N* sodium hydroxide solution, the same region no longer gave a purple Salkowski reaction. Instead, there was a red Salkowski reaction exactly like that given by indole-3-acetic acid. This red colour was more intense than the original purple, and had a greater spread of R_F value. Before sodium hydroxide treatment, no zone of the paper gave any trace at all of a red Salkowski reaction.

Both the red and the purple Salkowski reactions appeared to be given by two overlapping spots, indicating that the activity is not due to one compound only. A purple Ehrlich reaction given by the same region of the chromatogram also appeared to result from two overlapping spots.

The high activity relative to the indole-3-acetic acid controls shown in Fig. 2 has been noted in other experiments, but is not always reproducible.

These results were obtained with extracts from a Himalayan strain of maize, and also from a commercial variety, Northern Cross. The Salkowski and Ehrlich tests were carried out by the methods of Stowe and Thimann[2]. This work is continuing, and a further report will appear later. We wish to thank Profs. S. C. Harland and E. R. H. Jones for facilities and also Mrs. V. Shaw for carrying out biological tests.

Kathleen R. Farrar

Department of Chemistry,
The University,
Manchester 13.

Joyce A. Bentley

Marine Laboratory,
Aberdeen.

G. Britton
S. Housley

Department of Botany,
The University,
Manchester, 13.

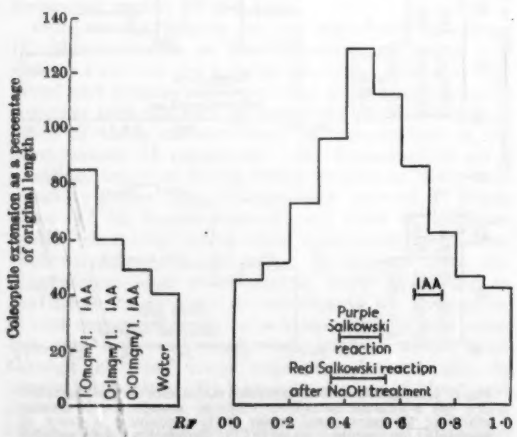


Fig. 2. Histogram of maize juice concentrate chromatographed in water-saturated *n*-butanol and tested by the *Avena* coleoptile straight growth test

[1] Bentley, J. A., and Housley, S., *Physiol. Plant.*, **7**, 405 (1954).
[2] Stowe, B. B., and Thimann, K. V., *Arch. Biochem. and Biophys.*, **51**, 499 (1954).

Influence of Introduction of a Fructosyl Group into Galactosylglucoses on their β-Galactosidase-inducing Activity

Introduction of a fructose group at the reducing carbon position of melibiose (to form raffinose) is known to abolish the β-galactosidase-inducing capacity of this α-galactoside in *Escherichia coli*[1,2]. The question arises whether this effect of the fructose group applies also to induction effected by β-galactosides. A study was therefore made of the β-galactosidase-inducing capacity of an homologous β-galactosidic oligosaccharide pair, namely, the pair lactose and α-lactosyl-β-fructofuranoside[3]. This last sugar, which will be referred to as 'lactsucrose', has a terminal β-galactosidic linkage, and is readily cleaved to galactose and sucrose both by *E. coli* *K*–12 β-galactosidase in solution and by a washed suspension of lactose-induced cells of this strain (Table 1). It proved, however, to be a negligible inducer of the β-galactosidase. Thus, the relative behaviour of lactose and lactsucrose resembles that of melibiose and raffinose in respect of the capacity to induce β-galactosidase in *E. coli* *K*–12. Moreover, it has also been shown recently that raffinose can be metabolized by melibiose-induced cells[4]. This indicates that raffinose is able to penetrate such cells. The cleavage of lactsucrose by lactose-induced cells indicates similarly that lactsucrose can enter these cells.

It is thus evident that the introduction of a fructosyl group at the reducing carbon of lactose results, as with melibiose, in a marked diminution of the ability of the disaccharide to serve as inducer of the β-galactosidase system. Such behaviour can be

Table 1. ACTIVITY OF *E. coli* β-GALACTOSIDASE TOWARDS LACTSUCROSE

Sugar in growth medium*	Inducer†	β-Galactosidase activity‡		Hydrolysis of the β-galactosidic linkage‖	
		Washed cells	Cell extract§	Lactose	Lactsucrose
Glucose	—	0·0	0·5	−	−
Glucose	lactsucrose	0·1	1·1	−	−
Glucose	lactose	9·8	182·5	+	+
Lactose	—	12·4	240·0	+	+

* Growth was conducted for 24 hr. in '56' synthetic medium containing 2 per cent sugar (ref. 1).

† Washed cells (in a concentration equivalent to 5 mgm. dry weight/ml.) were incubated 5 hr. at 37° C. in an induction medium (ref. 4) containing 0·02 *M* inducer.

‡ Expressed in terms of units of hydrolysis of *o*-nitrophenyl-β-galactoside (ref. 5) per mgm. dry weight.

§ Cells were mechanically disintegrated with glass beads in a Mickle vibrator. The supernatant solution given by centrifuging for 15 min. at 8,000*g* served as the enzyme solution.

‖ (+) indicates > 50 per cent and (−) < 2 per cent hydrolysis of substrate, both by cells and by their extract. The reaction mixture contained 0·1 *M* sugar, 0·05 *M* phosphate buffer *p*H 7·0 and fresh cells (5 mgm. dry weight/ml.) or enzyme solution (1 mgm. dry weight/ml.). After incubation at 37° C. for 6 hr. products of hydrolysis and decrease in the initial amount of substrate were observed by methods of paper chromatography and reducing sugar determination (ref. 3).

attributed to inability of the trisaccharide to reach the site of β-galactosidase induction, and/or a lack of inductive capacity proper. The permeation step is known to be controlled by a specific inductive system[7–9]. It is conceivable that raffinose and lactsucrose are incapable of inducing the permeation system, whereas cells induced by lactose possess it and hence are also penetrated by trisaccharides.

I wish to thank Prof. S. Hestrin for helpful advice.

GAD AVIGAD

Laboratory of Microbiological Chemistry,
The Hebrew University–
Hadassah Medical School,
Jerusalem, Israel.

[1] Monod, J., Cohen-Bazire, G., and Cohn, M., *Biochim. Biophys. Acta*, 7, 585 (1951).
[2] Lester, G., and Bonner, D. M., *J. Bact.*, **63**, 759 (1952).
[3] Avigad, G., *J. Biol. Chem.* (in the press).
[4] Landman, O. E., and Spiegelman, S., *Proc. U.S. Nat. Acad. Sci.*, **41**, 698 (1955).
[5] Lederberg, J., *J. Bact.*, **60**, 381 (1950).
[6] Lester, G., and Bonner, D. M., *J. Bact.*, **73**, 544 (1957).
[7] Rickenberg, H. W., Cohen, G. N., Buttin, G., and Monod, J., *Ann. Inst. Pasteur*, **91**, 829 (1956).
[8] Kepes, A., and Monod, J., *C.R. Acad. Sci., Paris*, **244**, 809 (1957).
[9] Pardee, A. B., *J. Bact.*, **73**, 376 (1957).

Demonstration of Some 'Masked' Lipids in the Oocytes of *Chrotogonus trachypterus* with Sudan Black *B* and Phenol 'Unmasking' Techniques

SUDAN black *B*, in spite of being the most specific and vigorous dye for the demonstration of lipids in tissue sections[1,2], has its limitations[3,4]. The cell inclusion believed to be lipid may not be coloured with sudan black *B*, because: (1) some lipids (like phospholipids) may escape from the tissue into the fixative by the process of emulsification[2,3]; (2) the lipids present have a high melting point and thus remain 'solid' in tissue sections[3]; or (3) the lipids present are 'masked' by proteins or other substances.

All these difficulties have been experienced in this laboratory during the histochemical investigation of the lipids in the oocytes of a number of animals[5]. The first one can always be eliminated by fixing the tissue in fluids containing free calcium ions[3] (like formaldehyde–calcium of Baker[2]), and by post-chroming; while the second difficulty can be overcome to a great extent by using the dye at 60° C. for longer periods (say 30 min.). But the lipids still remaining uncoloured have either a melting point much higher than 60° C. (for example, cholesterols and cholesteryl esters), or they may be 'masked'. The cholesterols and cholesteryl esters can always be detected by using specific histochemical tests[1,4,6]. It is the presence of 'masked' lipids which has troubled many previous workers.

Although the 'masked' lipids may be 'unmasked' and thus coloured with sudan black *B* by a simple treatment of the tissue sections with the various fat-solvents like acetone, ether, etc., which 'unmask' the lipids not removed by these solvents[5,7,8], yet such treatments are inadequate to 'unmask' the lipids in larger lipid bodies for which some special unmasking techniques recommended by Ciaccio[9] must be employed.

In the oocytes of *Chrotogonus* it has been found that a large number of sudanophil lipid spheres constantly give a 'ringed' or 'crescentic' appearance (that is, a dark sudanophil sheath enclosing a completely non-sudanophil core of various sizes) even when the sections of the material fixed according to the formaldehyde–calcium/postchroming technique[2] are coloured with sudan black *B* in propylene glycol[10] at 60° C. for 1 hr. These bodies give a completely negative reaction to all the tests for cholesterol and its esters[1,4,6], in spite of repeated attempts. This shows that if lipids are present in the core they must be 'masked'.

Ciaccio's[9] various techniques were tried to 'unmask' them. The methods, which include an 'unmasking' fixative, namely, Da Fano, Ramon y Cajal with or without potassium dichromate, did not prove successful. Of the two special 'unmasking' agents recommended, trypsin also was not so successful, but phenol gave excellent results. The material fixed in formaldehyde–calcium for 6 hr. was treated with 1 per cent phenol solution at 37° C. for 24 hr. The gelatin sections of this material when coloured with sudan black *B* did not reveal any 'ringed' or 'crescentic' appearance of the lipid bodies, which now coloured uniformly blue-black. This shows clearly that the lipids present in the uncoloured cores of the 'crescentic' and 'ringed' lipid spheres in the oocytes of *Chrotogonus* were 'masked' ordinarily by proteins; the presence of the latter is shown by the stainability of these lipid bodies with mercuric–bromophenol blue[11].

The kindness of Dr. S. Bradbury, Cytological Laboratory, Department of Zoology, University of Oxford, for sending the details of Ciaccio's techniques not available here, is gratefully acknowledged.

BRIJ L. GUPTA

Department of Zoology,
Panjab University, Hoshiarpur.
Panjab-India. Nov. 6.

[1] Pearse, A. G. E., "Histochemistry" (J. and A. Churchill, Ltd., London, 1954).
[2] Baker, J. R., *Quart. J. Micro. Sci.*, **87**, 44 (1946); **90**, 293 (1949); **97**, 161 (1957).
[3] Cain, A. J., *Biol. Rev.*, **25**, 73 (1950).
[4] Bradbury, S., *Quart. J. Micro. Sci.*, **97**, 499 (1956).
[5] Nath, V., *et al.* (in the press).
[6] Gomori, G., "Microscopic Histochemistry" (University Press, Chicago, 1952).
[7] Lovern, J. A., "The Chemistry of Lipids of Biochemical Significance" (Methuen and Co., Ltd., London, 1955).
[8] Casselman, W. G. B., and Baker, J. R., *Quart. J. Micro. Sci.*, **96**, 49 (1955).
[9] Ciaccio, G., *Boll. Soc. Biol. Sperl.*, **1**, 47 (1926).
[10] Chiffelle, T. L., and Putt, F. A., *Stain Tech.*, **26**, 51 (1951).
[11] Mazia, D., Brewer, P., and Alfert, M., *Biol. Bull.*, **104**, 57 (1953).

Influence of Time on the Reversible Association between Large Molecules: the Collagen System

It is increasingly evident that reversible interactions between macromolecules underlie many important biological functions; the actomyosin system is a classical example. Time-related changes in association between large molecules, which give rise to altered solubility and tensile strength (often associated with increased order), are well known to polymer chemists. It is suggested here that biological systems which rely on controlled intermittent association between specific macromolecules may, by increasingly long periods of association, become fixed in an irreversible or very slowly reversible state, resulting in impaired or lost function.

This communication reports an example of time-related changes in solubility of fibrils formed in relatively homogeneous solutions of collagen. Cold neutral salt extracts of fresh skin from growing animals contain collagen in solution which may be precipitated as an opaque gel composed of cross-striated fibrils, characteristic of the native tissue, by warming to 37° C.[1]. Purified collagen isolated from such extracts behaves in like manner[2]. Dilute acetic-acid extracts of collagen dialysed against neutral buffers can be gelled in a similar way. The effect of certain electrolytes and non-electrolytes on the rate of heat gelation has been briefly described[3].

It was observed in the course of these studies that the opaque collagen gels would occasionally be re-dissolved on cooling in the refrigerator. Fessler[4] has independently reported that one fraction of purified collagen obtained from rabbit skin by neutral salt extraction would, after precipitation by warming, redissolve on cooling. The investigations reported here revealed that one factor determining whether or not a gel would dissolve on cooling was the time it remained at the higher temperature.

Clear viscous solutions of collagen were prepared from calf dermis by extraction with 0·5 *M* acetic acid and dialysis consecutively against phosphate buffer, *p*H 7·6, $\Gamma/2 = 0{\cdot}4$, and sodium chloride, $\Gamma/2 = 0{\cdot}45$. Other samples were similarly prepared at physiological ionic strength and *p*H. These solutions are 'homogeneous' electrophoretically and in the ultracentrifuge. Partial composition of the dissolved collagen was reported earlier[1].

When warmed to 37° C. the opacity of the rapidly formed gel increased with time along a sigmoid curve as observed in a 'Klett' photoelectric colorimeter (No. 540 filter). Opacity, in this system, was a direct function of precipitated protein sedimentable at 80,000*g* for 1 hr. This was demonstrated by analysis of the supernatant solution and weighing of the dried, salt-free precipitates. Series of samples of the two neutral solutions described above were allowed to stand in a water-bath at 37° C. for varying time-intervals ranging from 10 min. to 48 hr. after reaching maximum opacity. At the end of each period the tubes were transferred to an ice-bath and the fall in opacity was measured at 1-min. intervals in the colorimeter. After the rate of decrease in opacity had diminished nearly to zero the samples were centrifuged cold at 80,000*g* for 1 hr. and the collagen-content of the supernatant solutions was determined by hydroxyproline analysis, and the dry salt-free precipitates were weighed. Results of a typical experiment using a collagen solution, sodium chloride 0·14 ionic strength buffered to *p*H 7·4 with 0·008 *M* phosphate, are shown in Fig. 1. On cooling a gel for 10 min. after it had reached maximum opacity at 37° C., there was a fairly prompt fall in opacity, levelling off at about 40 per cent of the control value. After 48 hr. of incubation, less than 10 per cent reduction in opacity was observed on cooling. The same phenomenon in a greater degree occurred in solutions of 0·45 ionic strength. The loss in opacity on cooling after 10-min. incubation was 100 per cent and after 48 hr. of incubation was less than 10 per cent.

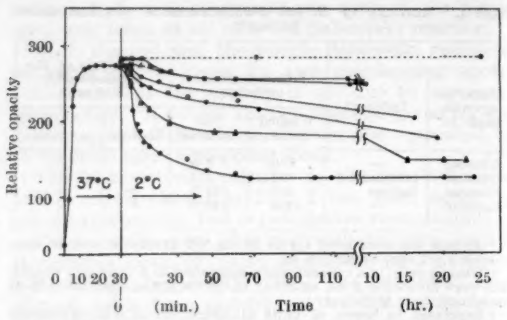


Fig. 1. Effect of cooling to 2° C. on the opacity of collagen gels incubated at 37° C. for different periods of time. Reading from the lowest curve upward: ●, 10 min.; △, 1 hr.; ◒, 3 hr.; ◓, 6 hr.; □, 24 hr.; ×, 48 hr.; ○ - - - - ○, 56 hr. (control, not cooled). Starting solution contained about 0·2 per cent calf-skin collagen dissolved in cold 0·14 *M* sodium chloride buffered with phosphate to *p*H 7·6

This phenomenon was also observed in cold saline extracts of fresh guinea pig skin which contained relatively high concentrations of a collagen fraction thought to be the precursor of the characteristic tissue fibrils.

Changes in solubility of the gels at acid *p*H as a function of time of previous incubation at neutral *p*H were also investigated. Between one and two weeks of incubation at 37° C. were required for the collagen gels to become almost completely insoluble in cold citrate buffer at *p*H 3·5.

It is improbable that the system reported here required interaction with non-collagenous components or enzymes. It is suggested that the increasing degree of irreversibility may result from the action of Brownian movement, allowing the associated molecules to find their most stable and sterically matched fit, thus permitting the establishment of more intermolecular cross-links.

The results of this study suggest the possibility that increasing time of association between biological high polymers may, at least in some instances, be contributing factors to ageing changes in structure and function in the organism.

This work has been supported by Grant No. *A* 90 (*C*7) of the Institute of Arthritis and Metabolic Diseases, U.S. Public Health Service, and an Established Investigatorship of the American Heart Association.

Jerome Gross

Department of Medicine,
Harvard Medical School, at the
Massachusetts General Hospital,
Boston, Mass. Nov. 14.

[1] Gross, J., Highberger, J. H., and Schmitt, F. O., *Proc. U.S. Nat. Acad. Sci.*, **41**, 1 (1955). Jackson, D. S., and Fessler, J. H., *Nature*, **176**, 169 (1955).

[2] Gross, J., *J. Exp. Med.*, **107**, 247 (1958).

[3] Gross, J., *Fed. Proc.*, **15**, 82 (1956).

[4] Fessler, J. H., *Fed. Proc.*, **16**, 37 (1957).

Isomorphous Replacement in the Kaolin Group of Minerals

It has been customary to classify the groups of layer silicate minerals as trioctahedral or dioctahedral according as all the octahedral ion sites in the lattice are occupied, or only two sites out of three. Virtually all minerals in the layer silicate groups fall clearly into one of these two divisions, the number of octahedral sites per unit cell occupied being usually nearly round numbers.

In the kaolin group the purely aluminous minerals, kaolinite, dickite and nacrite are dioctahedral members. Hallimond[1,2], Brindley[3,4] and Brindley and Youell[5] have shown that the chamosite present in sedimentary ironstones is a member of the kaolin group and that its molecular formula is very close to a trioctahedral structure, typically 2·9 octahedral sites out of every 3 being occupied by iron, magnesium or aluminium. There has been considerable recent evidence from microradiography[6], and chemical analysis and X-ray diffraction[7] that the composition of chamosite can vary between wider limits than had been supposed originally. Previous attempts to classify chamosite[8] have considered the Pauling structure for the kaolin and chlorite groups, trioctahedral members of which have a common empirical formula $X_3(SiAl)_2O_5(OH)_4$ [X = Fe,Mg,Al. . . .] representing half the unit-cell content of kaolin, and a quarter of the unit cell of chlorite. Agreement with this structure has not, in general, been very good. Other methods of classification[2,8] have used the oxide ratios in the formula of chamosite originally suggested by Zalinski[9]. This method has no justification from a structural point of view, but has indicated that for most chamosites derived from sedimentary ironstones the numbers of silicon and aluminium atoms in the structure are very nearly equal, although RO (where R = Fe or Mg) is often substantially lower than in Zalinski's formula.

It has recently been possible[10] to obtain a complete analysis of a chamosite from the chamosite–kaolinite (top) beds of the Northampton sand ironstone formation at Wellingborough. There were strong indications[6,7] that this specimen was particularly low in iron content, and analysis has confirmed these. The low iron content could not be accounted for by simple admixture of kaolinite. The structural formula, evaluated on a basis of 9(O + OH) ions in the formula, was :

$$\underbrace{Al_{1\cdot09}\ Fe^{2+}_{1\cdot30}\ Fe^{3+}_{0\cdot12}\ Mg_{0\cdot16}}_{2\cdot67}\ (Si_{1\cdot59}\ Al_{0\cdot41})\ O_{5\cdot15}\ (OH)_{3\cdot85}$$

This compares with the formula of a chamosite from the Main Ironstone beds of the same formation[5] at Corby :

$$\underbrace{Al_{0\cdot25}\ Fe^{3+}_{0\cdot01}\ Fe^{2+}_{1\cdot83}\ Mg_{0\cdot33}}_{2\cdot92}\ (Si_{1\cdot33}\ Al_{0\cdot67})\ O_{5\cdot02}\ (OH)_{3\cdot98}$$

It is clear that (*a*) the RO value is much lower than for the main ironstone chamosite, even when all iron is included as RO (1·58 against 2·07); (*b*) the occupation of octahedral sites is so low (2·67 against 2·92) that the Wellingborough specimen can scarcely be regarded as properly trioctahedral; and (*c*) the Si/Al ratio is still nearly 1. Replacement of tetrahedral silicon by aluminium is low, whereas the amount of octahedral aluminium is high; this represents a deviation towards the kaolinite composition.

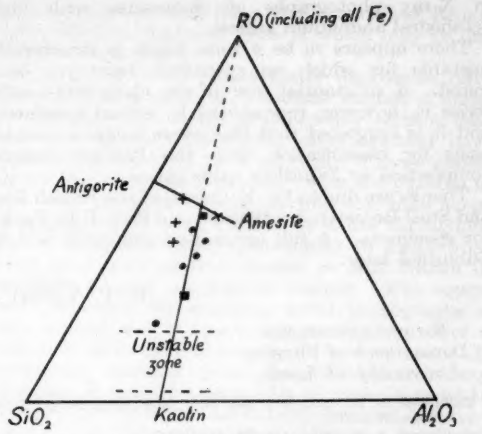


Fig. 1. Chamosite analyses: ■, present work; ●, Taylor; +, acid-extraction method (low aluminium); ×, lateritic origin (high aluminium)

This formula is so far from the Pauling structure composition that it seems necessary to seek some other method of classifying chamosite. It would appear that isomorphous replacement of iron and aluminium occurs between chamosite and kaolinite over a wide range of compositions, and that structures intermediate between dioctahedral kaolinite and the trioctahedral 'normal' chamosite are possible. Following Taylor[8], it is useful to present chamosite analyses as a triangular composition diagram (Fig. 1) based on SiO_2, Al_2O_3, and RO (the latter including all iron calculated as ferrous). On the basis that the silicon–aluminium ratios in chamosite are nearly 1, a line joining RO with the kaolinite formula ($2SiO_2$, Al_2O_3) should include all possible intermediates between chamosite and kaolinite. The chamosites from Wellingborough[10] and Corby[5] lie very close to this line and five analyses by Taylor[8] (although in some cases obtained by difference from materials containing only 30 per cent chamosite) are also in reasonable agreement with this line. Analyses quoted by Hallimond[2] and Taylor[8] made by the acid-extraction method (which tends to give low values for the aluminium content of the mineral) are well on the silica-rich side of the line, whereas a chamosite of lateritic origin[4] is, as may be expected, divergent in the aluminium-rich direction. Work by Grimshaw *et al.*, and Brindley, Robertson and Mackenzie has shown that a very small amount of substitution in the kaolinite lattice produces considerable disorder in the mineral. Very small amounts of iron present in place of aluminium produce a highly disordered fireclay and not a well-ordered kaolinite. This is in agreement with the much larger size of the ferric ion compared with the aluminium ion.

At the other end of the series, an iron-rich chamosite agreeing fairly well with the Pauling structure (as represented by the amesite–antigorite line) contains a high proportion of large ferrous and magnesium ions. Considerable substitution of small aluminium ions in the octahedral positions may occur without disrupting the lattice, although appreciable disorder is apparent

in X-ray photographs of chamosites with high octahedral aluminium values.

There appears to be a zone which is structurally unstable for which no specimens have yet been found. A substantial part of the chamosite–kaolin series is, however, represented by actual specimens, and it is suggested that this series forms a sounder basis for classification than the Pauling formula composition or Zalinski's oxide-ratios.

Thanks are due to Dr. E. Cohen, of the British Iron and Steel Research Association, and Prof. J. H. Taylor for specimens. A full account of this work will be published later.

R. F. Youell

X-ray Laboratories,
Department of Physics,
University of Leeds.

[1] Hallimond, A. F., Spec. Rep. Min. Res., Mem. Geol. Survey, Great Britain, **29** (1925).
[2] Hallimond, A. F., *Min. Mag.*, **25**, 441 (1939).
[3] Brindley, G. W., *Nature*, **164**, 319 (1949).
[4] Brindley, G. W., *Min. Mag.*, **29**, 502 (1951).
[5] Brindley, G. W., and Youell, R. F., *Min. Mag.*, **30**, 57 (1953).
[6] Cohen, E., *Metallurgia*, **41**, 227 (1949).
[7] Youell, R. F., *Nature*, **176**, 560 (1955).
[8] Taylor, J. H., "Petrology of the Northampton Sand Ironstone" Mem. Geol. Survey (H.M.S.O., London, 1949).
[9] Zalinski, E. R., *Neues Jahrb. Min.*, B, **19**, 40 (1904).
[10] Youell, R. F., thesis, University of Leeds (1955).

Dynamics of Chain Molecules in Dilute Solutions, with Special Reference to Non-Newtonian Viscosity

Several aspects of the phenomena appearing when chain molecules in dilute solutions are subjected to a velocity field seem to be satisfactorily explained by the theory which I have recently proposed[1]. In particular, the correct shape of the curve giving tan α (initial slope of extinction angle curve, as resulting from flow birefringence measurements) as a function of η_0 (viscosity of solvent) has been obtained; quantitative agreement has been observed between the calculated and the measured slope of the asymptote to the preceding curve, in the case of polystyrene; the correct dependence on molecular weight of the internal viscosity term B in tan α has been obtained (proportionality of B to the molecular weight, as established experimentally by Leray for polystyrene[2]); and finally it has become possible to understand some of the characteristics of non-Newtonian viscosity.

Furthermore, an expression of the intrinsic viscosity for molecules of low internal viscosity, valid in a wide range of velocity gradients, has recently been obtained[3]; this expression contains three molecular parameters which can all be determined experimentally: the molecular weight M, the zero gradient intrinsic viscosity $[\eta]_0$, and the ratio, B/M, which is characteristic of each kind of polymer.

These last results have been compared with the non-Newtonian viscosity measurements of Čopič[4] on a series of polystyrene fractions. The theoretical curves giving the intrinsic viscosity $[\eta]$ as a function of the velocity gradient G were computed, using the values of M and $[\eta]_0$ given by Čopič and the value of B/M deduced from flow birefringence measurements of Leray[2]. The agreement, though not perfect, is quite significant[3].

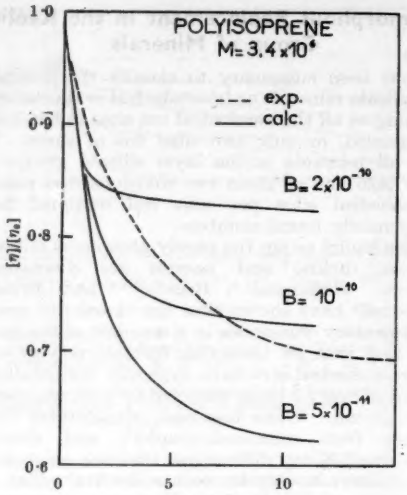


Fig. 1

Here, I wish to make the comparison with the measurements of Golub on a series of polyisoprene fractions[5]. No flow birefringence data are available in this case; therefore it is not possible, as in the case of polystyrene, to compute the $[\eta]$ versus G curves from the theory, not using any adjustable parameter.

I have first computed three theoretical curves for polyisoprene 1*c*, giving B/M respectively the values 5×10^{-11}, 10^{-10}, 2×10^{-10}. In Fig. 1 it can be seen that a reasonable agreement with the experimental curve is obtained taking $B/M = 10^{-10}$.

The theoretical curves for the samples 1*a*, 3*a* and 3*b* have then been computed (taking B/M as a constant). The comparison with the experimental curves is made in Fig. 2 for the samples 3*a* and 3*b*: the agreement is quite good. For sample 1*a* the agreement is of the same type as for 1*c*; the curves relative to 1*a* are not represented here.

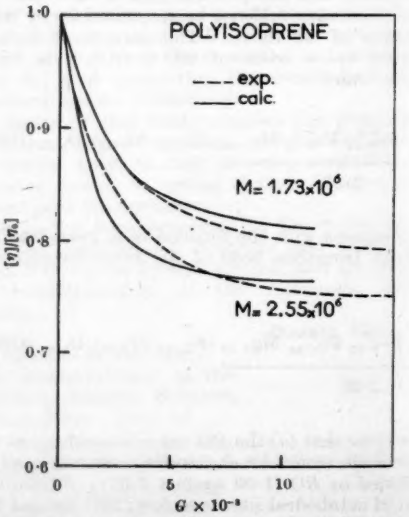


Fig. 2

Deviations like those observed on Fig. 1 are not surprising, since the conditions of validity of the theory are not rigorously fulfilled (the internal viscosity of polyisoprene is a little too high), and since the theory becomes insufficient when G approaches infinity.

Thus the theory explains a large number of facts, and is well suited to describe many essential features in non-infinitely small gradients. It is hoped that new possibilities of the hydrodynamic methods will follow; it is already possible to obtain the parameter B, and therefore the internal viscosity of the molecule, from one single $[\eta]$ versus G curve.

ROGER CERF

Centre de Recherches sur
les Macromolécules,
6 rue Boussingault,
Strasbourg.
Nov. 22.

[1] Cerf, R., *C.R. Acad. Sci., Paris*, **240**, 531 (1955); **241**, 496, 1458 (1955); **243**, 1875 (1956); **244**, 456 (1957); *J. Polymer Sci.*, **23**, 125 (1957); **25**, 247 (1957).
[2] Leray, J., *C.R. Acad. Sci., Paris*, **241**, 1741 (1955).
[3] Cerf, R., *C.R. Acad. Sci., Paris*, **245**, 1062 (1957).
[4] Copič, M., *J. Chim. Phys.*, **54**, 348 (1957).
[5] Golub, M., *J. Phys. Chem.*, **60**, 431 (1956).

A Survey of Terrestrial Craters

The domination of the Moon's surface by circular crater-like features raises the question as to whether this is a phenomenon peculiar to the Moon or whether it has significance for the solar system as a whole, including the Earth. The latter view has been urged by Baldwin[1] and others[2,3], who conclude that the lunar craters are due to meteorite impact and that the Earth has suffered a similar bombardment of which known meteorite craters are the residual evidence.

Baldwin's correlations are on the whole rather convincing, but the data on terrestrial meteorite craters available at the time his work was done were statistically less impressive than might be desired. Although widely distributed over the Earth, specimens being found in Europe, Asia, Africa, Australia and North and South America, only 17 craters or clusters of craters were known prior to 1950. While some of the clusters contained many pits, they were for the most part small, and only ten individual craters were known to be more than 100 metres (330 ft.) in diameter, the limit of size which can be observed on the Moon (Kuiper, G. P., private communication). In addition, all these craters belong to a recent geological epoch, whereas there is evidence pointing to a wide distribution of ages among lunar features.

Discoveries made during the past seven years have provided appreciable extensions to the data in the directions of numbers, diameters and ages of craters. These include the Chubb or New Quebec Crater, 3·4 km. diameter (Recent)[4], the Talemzane Crater, 1·8 km. diameter (Late Pliocene or early Quaternary)[5], the Brent Crater, 3·7 km. diameter (Early Paleozoic)[6], the Holleford Crater, 2·4 km. diameter (Early Paleozoic)[7] and the crater at Deep Bay, Saskatchewan, diameter 13·7 km. (Late Mesozoic)[8]. In addition, two large circular arcs have been noted in Canada, the Belcher Islands arc in Hudson Bay, 400 km. diameter, and the arc containing Prince Edward Island and outlined by the neighbouring coasts of Nova Scotia and New Brunswick[9], 300 km. diameter, both of which may be due to meteorite impact.

Most of the more recent crater discoveries have been made as a result of systematic studies of aerial photographs, and it seems reasonably certain that some of the most interesting objects would have remained in obscurity for a long time if these photographs had not been available. It is the purpose of this communication to suggest that the wide aerial photographic coverage which now exists throughout the world represents an opportunity to make what could be a decisive contribution to this branch of astronomical and geophysical science. The equipment necessary for studying aerial photographs is very modest, and a determined worker can cover a relatively large area of country in a short time. If systematic searches could be initiated in many countries, it would be reasonable to expect a trend to emerge which would go far to settle the still unsolved problem of the relationship between terrestrial meteorite craters and the astonishing array of circular objects covering the surface of the Moon.

C. S. BEALS
(Dominion Astronomer)

Dominion Observatory,
Ottawa.

[1] Baldwin, R. B., "The Face of the Moon" (Univ. of Chicago Press, 1949).
[2] Kuiper, G. P., *Proc. U.S. Nat. Acad. Sci.*, **40**, 1096 (1954).
[3] Urey, H., "The Planets" (Yale Univ. Press, 1952).
[4] Meen, V. B., *J. Roy. Astro. Soc. Canada*, **44**, 169 (1951).
[5] Karpov, R., *Meteoritics*, **1**, 31 (1953).
[6] Beals, C. S., Ferguson, G. M., and Landau, A., *J. Roy. Astro. Soc. Canada*, **50**, 207 (1956).
[7] Beals, C. S., *Sky and Telescope*, **16**, No. 11 (1957).
[8] Innes, M. J. S., *J. Roy. Astro. Soc. Canada*, **51**, 235 (1957).
[9] Willmore, P. L., and Scheidegger, A. E., *Trans. Roy. Soc. Canada*, **50**, 21 (1956).

Etch Figures on Sodium Chloride Crystals

GYULAI and his co-workers have for some years been investigating the properties of alkali halide crystals. In the course of etching sodium chloride and rock salt crystals I have made some observations which may throw light on the dislocations supposed to exist in real crystals.

Cleaved crystals of sodium chloride were etched in alcohol mixed with a small quantity of formaldehyde. After etching for a few minutes, that part of the sample which was in contact with the container revealed etch-figures, shown in Figs. 1 and 2. The examination and photography of these samples was carried out in transmitted light, with an '*L*-Stative' Zeiss microscope. The etch-figures are most instructive. The growth layers, and the lamellar structure of the crystals show up clearly. On the single growth steps right- and left-handed spirals and their various combinations have become visible. The single spirals are squares with rounded corners, their orientation corresponding with the cubic edges of the crystal. The direction of observation was (001).

The photomicrographs give a detailed picture of the etched (001) plane. The etch-figures are similar to those obtained by A. R. Verma[1] on silicon carbide. Voltava and Amelinckx[2] obtained spiral figures on the surfaces of sodium chloride crystals by thermal etching. The spirals and their combinations obtained by the use of etching reagents are, however, clearer,

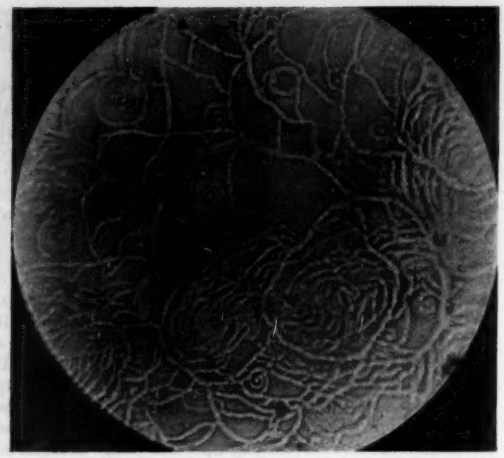


Fig. 1. Surface of a sodium chloride crystal after etching in alcohol +formaldehyde for 30 min. × 500

more easily obtained and are durable in dry air. The etching method described here may be applied to synthetic as well as to natural crystals, and with appropriate modification may also be used for potassium chloride crystals. Investigations in this direction are being carried out.

Another observation has been made on deformed crystals of sodium chloride. Synthetic blocks of sodium chloride a few millimetres in length and diameter were slightly deformed by bending. After etching a deformed crystal for 0·1 sec. in glacial acetic acid very regular etch-pits were obtained on every side of the sample. The etch-pits are regular, square-shaped concave pyramids. These observations were also made with a Zeiss '*L*-Stative' microscope, with a magnification of about 100, in transmitted light. The etch-pits are located on the (001) plane as seen from the direction of the bending axes on the (110) slip planes. The etch-figures are grouped in more or less dense strips. It is, however, unnecessary to deform the sample, because cleavage itself initiates slips, which seems to be unavoidable. When preparing the sample by cleaving it on six sides,

Fig. 2. Cleaved surface after etching for 0·1 sec. in glacial acetic acid. × 250

deformations are produced in the crystals. Thus every surface examined contained systems of two perpendicular bands and two bands inclined at an angle of 45° to the (100) direction. These bands consist of square etch-pits and in some cases they may be observed individually. According to the observations of W. Dekeyser, J. J. Gilman and W. G. Johnston[3] and others, such etch-pits originate at the ends of dislocations emerging on the surface. Taking into consideration the potential mobility of the dislocations, the network of the 45°-bands may be interpreted as containing edge-dislocations, and the 90°-orthogonal network as containing screw-dislocations. Etching in glacial acetic acid provides a very quick method for investigating grain boundaries. After a short etching time, the grain boundary becomes readily visible as a region densely packed with distorted etch-pits.

It is interesting to observe that the edges of the cleavage lamellæ are attacked by the reagent only after a considerably longer period.

Further investigations are in progress.

B. Jeszenszky

Institute of Experimental Physics,
Technical University,
Budapest.
Sept. 9.

[1] Verma, "Crystal Growth and Dislocations" (Butterworths Scientific Publications, London, 1953).
[2] Voltava and Amelinckx, *Naturwiss.* (1954).
[3] Dekeyser, Conf. on Defects in Crystalline Solids, Bristol (July 1954). Gilman and Johnston, *J. App. Phys.*, **27**, 1018 (1956).

Relativistic Aberration Formula

It has been stated that "the relativistic aberration formula, $\tan\theta' = \sin\theta(1 - v^2/c^2)^{1/2}/(\cos\theta + v/c)$ ... deviates from the equation ... of the ether theory by quantities of second order only, a deviation which is negligible compared with the present accuracy of the measurements"[1]. The aberration is also referred to as a "displacement, $\theta - \theta'$"[2].

The angles θ, θ', however, which appear in the formula, are measures in different frames of reference, and subtraction of them is meaningless observationally. The 'displacement', as measured by the observer, would be $\theta - \theta''$, where θ, θ'' give, respectively, the apparent and actual directions of the source in the frame of the observer, simultaneously for him. But in that frame, since the source has moved vt while the ray has moved ct, t being the time of transit from source to observer, we have $\tan\theta'' = \sin\theta/(\cos\theta + v/c)$, exactly as in the classical theory for an observer at rest in the ether. It is obvious, in fact, that there would be no deviation between the classical and relativity theories for such an observer, since no transformation of co-ordinates is involved.

If it is the source which is at rest in the ether, the classical theory requires us to superpose on the picture relative to the observer an ether drift of v in the direction of the source's velocity. The velocity of the light-ray being now approximately $(c + v\cos\theta)$ relative to the observer, by the same argument we find $\tan\theta'' = \sin\theta/\{\cos\theta + v/(c + v\cos\theta)\}$, which gives a second-order deviation between the theories amounting to twice the deviation now accepted. In general, with an ether drift of V (not commensurate with c) away from the observer, the deviation

is (vV/c^2) tan θ instead of $\frac{1}{2}(v^2/c^2)$ tan θ, as given by the accepted formula.

J. M. WHITEMAN

Department of Mathematics,
University of Cape Town.

[1] Møller, C., "The Theory of Relativity", 62 (Oxford, 1952). Bergmann, P. G., "Introduction to the Theory of Relativity", 37, formulæ 4.19, 4.20 (New York, 1942).
[2] Dingle, H., "The Special Theory of Relativity", 51, 2nd edit. (London, 1946).

Random Time

IF the point of the hand of a clock moves along the circumference of the unit circle with constant angular velocity ω, then the time measured is determined by the position:

$$\exp i\omega t \tag{1}$$

This formulation depends, however, on the abstraction of a 'uniform time', a concept which, after Mach's critique, fails to be meaningful and cannot be made legitimate even if a built clock is replaced by a clock determined by the gyrations of the Earth (or, for that matter, of a galaxy). Clearly, the conceptual difficulty remains unaltered if, instead of astronomical clocks, recourse is had to electron clocks—those supplied by electromagnetic or spectroscopic measurements.

Actually, the irreducibility of the vague notion of what is the 'uniformity' of the independent variable t (classical or relativistic) is not merely an epistemological issue. For 'time' can be defined only by measurements, and the measurements can be carried out only on the movements of instruments (clocks, planets, electrons), whereas every physical object and its observations are necessarily subject to imperfections or, at best, to statistical fluctuations. What is more, the same holds for every physical law as well (by physical law we understand here differential equations in which t is an independent variable). The object of this communication is the harmonic analysis of the simplest (and, in a certain mathematical sense, uniquely determined) model of the statistical fluctuations of 'time' itself.

If $f(t)$ is defined for $-\infty < t < \infty$ as a function for which the auto-correlation:

$$F(t) = \lim_{T\to\infty} \frac{1}{2T} \int_{-T}^{T} f(t+s)\bar{f}(s)ds \tag{2}$$

exists as a continuous function for $-\infty < t < \infty$ (the bar refers to complex conjugation), then $F(t)$ is necessarily of the form[1]

$$F(t) = \int_{-\infty}^{\infty} \exp itu\, d\varphi(u) \tag{3}$$

where $\varphi(u)$ is a certain bounded function satisfying $d\varphi(u) \geqq 0$.

If $f(t)$ is chosen to be the function (1), then it is identical with $f(t+s)\bar{f}(s)$ for every s. Hence (2) shows that $F(t) = f(t)$ if $f(t) = \exp i\omega t$, which means, by (3), that the distribution function φ (representing the spectral energy) is a Dirac distribution, consisting of a single saltus: $\varphi(u) = 0$ for $-\infty < u < \omega$ and $\varphi(u) = 1$ for $\omega < u < \infty$. Clearly, the statistical fluctuations of time, referred to above, will have to dissolve the resulting infinite concentration (represented by a discrete, sharp spectral line) at $u = \omega$. Let the fluctuations be realized as follows:

Add to the t of (1) the function $ax(t, \alpha)$, where a denotes a positive constant and $x(t, \alpha)$ is a normalized function as defined in the theory of differential space[2]. Then, since (1) or $f(t) = \exp(i\omega t)$ is replaced by:

$$f(t, \alpha) = \exp(i\omega\{t + ax(t, \alpha)\}) \tag{4}$$

where x is real-valued and α is a parameter (to be averaged over the interval $0 \leqq \alpha \leqq 1$), the function (2) becomes:

$$F(t, \alpha) =$$

$$\exp i\omega t \lim_{T\to\infty} \frac{1}{2T} \int_{-T}^{T} \exp(i\omega a\{x(t+s, \alpha) - x(s,\alpha)\})ds \tag{5}$$

Here α is a point (not contained in a set of measure zero) on the integration-range of differential space.

In view of the Gaussian definition of measure in differential space, it can readily be concluded from the ergodic theorem[3] that, for almost all values of α, the time-average which multiplies $\exp i\omega t$ in (5) is identical with the corresponding space-average in differential space, and, therefore, with the integral

$$\int_{-\infty}^{\infty} \exp(i\omega av)(2\pi|t|)^{-1/2} \exp(-\tfrac{1}{2}v^2/|t|)dv$$

But this is Laplace's integral, the value of which is known to be $\exp(-\frac{1}{2}\omega^2a^2|t|)$. If this evaluation is substituted in the representation (5) of the function $F(t, \alpha)$, a function which, being independent of α (for almost all α), can now be denoted by $F(t)$, and if the result is compared with (3), it follows that:

$$\int_{-\infty}^{\infty} \exp itu\, d\varphi(u) = \exp i\omega t \exp(-\tfrac{1}{2}\omega^2a^2|t|)$$

This can be written in the form

$$\int_{-\infty}^{\infty} \exp itu\, d\varphi(u-\omega) = \exp(-\rho|t|) \tag{7}$$

where

$$1/\rho = 2/(\omega a)^2 \tag{8}$$

Finally, the integral of $\exp(itu)/(1+u^2)$ over $-\infty < u < \infty$ is known to be $\pi\exp(-|t|)$. Hence it is clear from (7) that the spectral distribution, instead of being concentrated (as for (1) above) at the single frequency $u = \omega$ of infinite density, is spread out continuously over the entire range $-\infty < u < \infty$. In fact, the density of probability in the resulting distribution is seen to be

$$\frac{d\varphi(u)}{du} = \frac{1}{\pi} \frac{1}{1+(u-\omega)^2} \tag{9}$$

if either the value of a in (4) or the unit of length on the u-axis is so chosen that the constant (8) becomes 1.

It should be mentioned that the result (9) is precisely the *elementary* distribution of Cauchy, a

stable distribution of infinite standard deviation, already encountered by P. Lévy in his work on normal stochastic processes[4].

Norbert Wiener

Massachusetts Institute of Technology.

Aurel Wintner

The Johns Hopkins University.

[1] Wiener, N., "The Fourier Integral and Certain of its Applications", Chapter 4 (Camb. Univ. Press, 1933). For further developments, cf. Wiener, N., and Wintner, A., *J. Math. and Phys. (M.I.T.)*, 17, 233 (1939); and Wiener, N., and Wintner, A., *Amer. J. Math.*, 63, 796 (1941).
[2] Wiener, N., *Acta Math.*, 55, 117 (1930).
[3] Birkhoff, G. D., *Proc. U.S. Nat. Acad. Sci.*, 17, 650 (1931).
[4] Lévy, P., *Compositio Math.*, 7, 283 (1939).

Contraction of Time and Protoplasm

In recent discussions concerning the clock paradox, biological time is mentioned but dismissed as of little consequence in the matter[1]. Contrary to this view and regardless of the solution finally found acceptable in the clock paradox, the entire problem really centres in biological time. Whether we accept or reject a completely mechanistic view of protoplasmic processes and therein compare them to an actual clock in the sense of their mechanical workings, or believe in other processes, in addition to the mechanistic view, the statement that biological time is the centre of the matter still holds. No one has ever shown that biological processes are identical in their functional attributes to the mechanical universe envisaged by the physicists. If biological time is not identical to the physicists' time then asymmetrical ageing is meaningless, so far as biological phenomena are concerned. One of the most outstanding physicists, upon analysing the meaning of life, gives an opposite view. Schrödinger[2] states: "from all that we have learnt about the structure of living matter we must be prepared to find it working in a manner that cannot be reduced to the ordinary laws of physics". I take it from this statement, and my own experience of more than twenty-five years of working with living materials, that the laws which control protoplasm are of a different order of integration from those of ordinary physics. This is not surprising in view of its functional complexity.

If we admit the postulates of the relativity theory apply to time–space factors, using a mechanical clock to reveal the asymmetry of the result, this is one thing; if we use a human or other living organism to reveal the result, the effects may predictably be an entirely different matter. The space–time dilatation is most profoundly related to biological processes in two obvious aspects: (1) all primary data upon which the concept is based are biologically sensorially derived; (2) if time–space factors are to show asymmetrical age differences in the stay-at-home versus the space traveller, then the biological processes of growth and ageing are involved.

Aspect (1), of course, looks directly into the whole problem of reality and causality. If as Russell[3] and others think, namely, that causality originates in perception, we can scarcely escape the implication of this in our reasoning about time dilatation and biological time. The two are closely linked. While the final status of biological time is probably in as confused a state[4] as is the clock paradox, we can, in a sense, deal with it in a more direct experimental manner.

The involvement of biological factors of ageing and growth processes in the relativistic contraction of time is very real. Biological matter has to exist not only in a physical inertial frame, of some sort, but also in some kind of time continuum. The problem here is whether or not biological material (protoplasm to be specific) is in any way affected as a function of velocity, or if it is affected in any way, or if the narrow bios zone which is an absolute necessity for survival of protoplasm also includes absolute velocity. (The discussion disregards acceleration, deceleration effects upon the intact animal which are in part understood.)

McCrea[5] mentions biological time but denies its importance through the route of making the difference between biological time and clock time arbitrarily small. He says that biological processes need not be standardized with a standard clock accompanying the system. This is the heart of the problem: is not our entire interpretation of time sense biologically conditioned? The question must be asked whether all the biological processes depend upon rate of travel of protoplasm The supposition is that growth processes will be retarded or actually reversed, yet everything we know about protoplasmic processes denies that this is possible. Living processes may be temporarily suspended under certain conditions, or slowed down, but the range of this is extremely narrow. Since all other factors, which maintain protoplasmic integrity, are of a narrow limited range, may not the same be true of the function of velocity? The interesting thing is that it does not seem to have occurred to anyone that experiments might well be performed in this connexion.

The implication in the work on asymmetric ageing is astounding since the physicists predict that increased velocity will slow up or reverse what are major biological functions, namely, the ageing and growth functions. It is as though they believe both of these are simply physical models of systems identical in final analysis to a clock. Without getting into the idea of entelechy, it suffices to state that while we may accept the physicist's view of biological materials we cannot deny that the order of organization of protoplasmic processes may be such that the ultimate upper integrative level of biological processes may function in a manner entirely different from the materials thus far tested on the electron–proton level.

Taking the other facet of the argument, assuming that the physicists are correct, and that growth and ageing processes are connected with velocity in the fashion they assume, it would be of extreme importance to determine this, since by utilization of the velocity factor we could modify living processes almost at will. Again an experimental approach is called for in order to secure the answer.

Anderson Nettleship

Medical Center, University of Arkansas, Little Rock.

[1] Dingle, H., *Nature*, 177, 782, 785 (1956); 178, 680 (1956); 179, 865, 1242 (1957).
[2] Schrödinger, E., "What is Life?" (Cambridge Univ. Press, 1951).
[3] Russell, Bertrand, "The Analysis of Matter" (Dover Pub. Inc., New York, 1954).
[4] Du Nouy, Le Comte, "Biological Time" (Methuen and Co., Ltd., 1936).
[5] McCrea, W. H., *Nature*, 179, 909 (1957).

Propagation of Ultrasonic Waves in Liquids

We had observed[1] that the heat generated by ultrasonic waves in carbon disulphide and in other liquids, such as toluene and xylene, is not in conformity with their widely divergent absorption coefficients as measured by other (acoustic) methods. To clarify the point further, we have made a set of measurements with the type of apparatus described in ref. 1.

Working at about 32° C., it was observed with a particular apparatus that the total power generated by a vibrating quartz crystal, placed inside a container with kerosene oil or xylene, was 9 watts, as obtained from the steady temperature difference between the container and the circulating water outside. Energies were also measured in a trap-vessel, placed at a definite distance from the quartz; on both sides of it when the quartz was in the middle of the container, and on one side only when the quartz was near the end. It was observed that the energy propagated forward from the quartz placed near one end was 25 per cent higher than from the other position, where it was propagated equally on both sides. The energy propagated forward was thus found to be 5·6 watts only.

Measurements were taken with trap-vessels of different sizes, placed at different distances from the quartz, which was near the end of the container. The liquids outside and inside the trap vessels were varied. It will be seen from Table 1 that α/f^2, obtained from the variation of power with distance, drops from a comparatively large value near the quartz to a value of approximately the same order as that obtained by other methods.

A direct measure of the energy incident on a trap-vessel, passing through kerosene oil and out on the farther side, was obtained by placing the trap in a closely fitting glass container, 5 cm. from the quartz. The container was thus virtually partitioned into three thermostatic chambers, except for the communicating mica windows. It was cooled by water circulating outside. The power on the farther side of the trap-vessel was measured from the temperature difference between it and the circulating water. This is a measure of the vibrational and turbulent energy as well as of the heat passing out of the trap, having regard to the temperatures on the two sides. We obtained a value of 4·5 watts, incident on the trap-vessel transmitted through the windows, irrespective of the liquid inside.

The variation of the measured power with distance has already been noted. It shows that when the incident energy at 5 cm. from the quartz is 4·5 watts, the power output at the quartz is not less than 5·6 watts. It accounts for the energy that was found to be propagated from the quartz, and it appears that the mica windows do not reflect any detectable energy.

The power measured in the different vessels, placed at 5 cm. from the quartz, gives, having regard to the incident energy, a very high absorption coefficient for liquids known to have low absorbing power.

The coefficients, in the form α/f^2, so derived are shown in Table 1 (column 7).

The higher absorption of ultrasonic waves near the source as determined by energy variation with displacement is to be associated with the inharmonic character of the waves observed by Hubbard[2]. Moreover, the excessively high absorption in the

Table 1

Liquids (*a*) outside (*b*) in trap	Distance from quartz (cm.)	Trap vessel size (cm.)	Power in traps (watts)	Incident power at 5 cm. (watts)	$\frac{\alpha}{f^2}\times10^{17}$ from displacement, liquid (*a*)	$\frac{\alpha}{f^2}\times10^{17}$ from incident energy, liquid (*b*)
(*a*) Kerosene (*b*) Kerosene	2	1	1·5	4·5	408	1,722
	5	1	1·2			666
		5	2·0			400
		15	3·0		200	
	10	1	1·0			
	15	1	0·85		180	
(*a*) Xylene (*b*) Xylene	2	1	1·4	4·5	583	1,444
	5	1	1·02			600
		5	1·9			270
		15	2·3		255	
	10	1	0·81			
	15	1	0·70		166	
(*a*) Kerosene (*b*) Carbon disulphide	5	1	2·0	4·5		3,330
		5	2·3			800
		15	2·4			325

1-cm. trap-vessel, for low-absorption type liquids, was not caused by reflexion from the mica. It has also been confirmed that diffraction by the aperture does not account for this effect. The observations appear capable of explanation on the basis of a phase retardation of the components of the inharmonic wave-form, introduced by the first mica window. They become progressively out of step and are thus prevented from being fully transmitted by the second mica window. Even in unobstructed liquids, this mechanism appears to operate to a small extent and to account for the larger loss of energy at smaller distances. It supports the conclusion that the excessive absorption of ultrasonic waves noted in carbon disulphide and benzene (and possibly also the discrepancy from the Stokes absorption coefficient in other liquids) is due to a mechanism which produces more or less effectively a confusion in phase relationship during propagation, in the same way that the mica window does for low-absorption liquids such as xylene. It thus appears that wave trains in liquids can lose energy, as if by scattering, in a manner similar to that noted by Richardson[3] in the case of gases.

A. K. Dutta
K. Samal

Ravenshaw College,
Cuttack.

[1] Dutta, A. K., and Samal, K., *Nature*, **174**, 976 (1954); **179**, 95 (1957).
[2] Hubbard, J. C., Fitzpatrick, P. A., Kankovsky, B. T., and Thaler, W. J., *Phys. Rev.*, **74**, 107 (1948).
[3] Richardson, E. G., *Proc. Roy. Soc.*, A, **146**, 56 (1934).

Protection of Fœtuses against Radiation Injury

The protection against a lethal dose of radiation, conferred on mice by grafts of hæmopoietic tissue, is being attributed to repopulation of homologous tissues of the host by cells from the graft[1]. This does not, however, exclude the possibility that such grafts might also be supplying a subcellular factor able to contribute to the recovery of the host.

To test the latter possibility, female mice of strain *A* were given, on the fourteenth day of pregnancy, a dose of X-rays amounting to 275 r. Immediately following this, and always under ether anæsthesia, they were splenectomized. One group was treated no further, while each animal of a second group was

Table 1. Weights (gm.) of Surviving Young at the Age of 37 Days

Young from 'ungrafted' mothers		Young from 'grafted' mothers	
Litter	Weight	Litter	Weight
A 1	5·0	*B* 1	13·6
	4·0		15·5
			14·0
A 2	6·0		
	5·5	*B* 2	11·6
	4·9		11·1
	5·2		12·3
			8·0
A 3	5·9		6·4
	4·0		
		B 3	11·6
A 4	4·2		13·9
	12·4		12·9
			15·0
		B 4	11·8
			14·0
			14·0
			12·5
			12·5
Mean	5·7±2·3	Mean	12·4±2·2

given an intraperitoneal graft of six macerated livers from 18-day old fœtuses of the same strain.

Only six out of ten animals of the first ('ungrafted') group gave birth to litters. Dissection showed that resorption of fœtuses had occurred in the others. The six litters born included several still-born as well as twenty-six live young. Of the latter, twelve died within a week and another four died before weaning, having reached a weight of no more than 2–4 gm. each. The weights of the remaining ten at the age of 37 days are given in Table 1.

In contrast, all four females of the second ('grafted') group gave birth to litters, with a total of twenty-two live young and six still-born. Another five died before weaning; but the remaining seventeen developed to normal adult weight.

It would appear from these observations that the grafting of fœtal livers into the peritoneal cavity of pregnant mice subjected to irradiation improves the chances of the fœtuses to survive and enables those actually surviving to reach an adult weight twice as great as that of young from irradiated but ungrafted mothers. Experiments are being continued, based on the hypothesis that the effect of the graft is due to a humoral factor from it reaching the fœtuses *in utero*.

I am indebted to Prof. C. H. Waddington and Dr. D. Falconer for facilities provided at the Institute of Animal Genetics, Edinburgh, and to the Melville Trust for a Research Fellowship.

E. M. Pantelouris

Zoology Department,
Queen's University,
Belfast.
Oct. 30.

[1] Bond, V. P., and Cronkite, E. P., *Ann. Rev. Physiol.*, **19**, 299 (1957).
[2] Rugh, R., and Clugston, H., *Science*, **123**, 28 (1956).

Hæmoglobin Types in Ox and Buffalo

Studies of ox and buffalo hæmoglobins by the technique of electrophoresis have been made by several workers. Geiger[1], using a technique of cataphoresis, resolved ox hæmoglobin into a component moving anodally and a component moving cathodally at a neutral reaction, and noticed that the two components had different oxygen-dissociation properties. Reiner, Moore, Lang and Green[2] and Munro and Munro[3], using the moving-boundary technique, resolved ox hæmoglobin into two components at an acid reaction. Using paper electrophoresis, Cabannes and Serain[4] found two varieties of hæmoglobin (which they called α and β) in 270 samples of North African oxen (α alone in 76·3 per cent, α plus β in 20·7 per cent, α alone in 4·0 per cent). Giri and Pillai[5] studied buffalo hæmoglobin on agar electrophoresis and resolved it into two components.

In the course of an investigation of human and animal hæmoglobin by the silicone-treated glass-plate electrophoresis technique[6] using veronal buffer *p*H 8·6 (ionic strength 0·06) and citrate buffer *p*H 6·5 (ionic strength 0·05), it became possible to study the hæmoglobin patterns in 27 oxen (*Bos banteng*) and 28 buffaloes (*Bubalus bubalis*) imported into Singapore from Bali.

In the ox, three types of hæmoglobin were detected (*A*, *B* and *C*, Fig. 1) and five patterns were obtained: *A* alone (nine cases, 33·3 per cent), *B* alone (eight cases, 29·6 per cent), *C* alone (one case, 3·7 per cent). *A* plus *B* (four cases, 14·8 per cent), and *A* plus *C* (five cases, 18·4 per cent). *A* was found in eighteen cases (66·6 per cent), migrated at a faster rate than normal adult hæmoglobin and is probably identical with Cabannes and Serain's β. *B* moved more slowly than *A*, very similar to normal adult hæmoglobin, and is probably identical with Cabannes and Serain's α. *C* moved faster than *A*. All three types moved anodally at *p*H 8·6, *A* and *B* being well separated, *A* and *C* less so. At *p*H 6·5 all three types moved cathodally, their relative migrations being reversed.

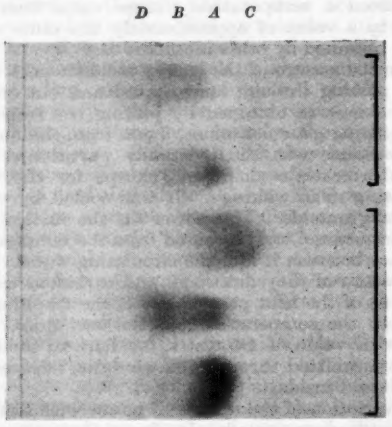


Fig. 1. Electrophoresis (Whatman 3*MM* filter paper) at *p*H 8·6 (ionic strength, 0·06), 360 V., 20 mA., 5–6 hr.

In the buffalo two components with mobilities identical with those of ox *A* and *B* were found in 27 cases (96·4 per cent), while in one case a small amount of a third component, moving more slowly than *B*, was noticed.

The one-minute alkali denaturation values[6] for the ox bloods varied between 70–90 per cent (average 75·2 per cent) and for buffalo bloods 64–90 per cent (average 79 per cent). Alkali denaturation patterns over 4 hr. were indistinguishable when ox *A*, *B*, *C*, *AB*, *AC* or buffalo bloods were used, indicating that several alkali-resistant hæmoglobins may occur in the same species.

Absorption spectra for ox *A*, *B* and *C* were determined between 2000–5000 A. They were found to be identical in all three cases.

FRANCIS VELLA

Dept. of Biochemistry,
Faculty of Medicine,
University of Malaya,
Singapore.
Oct. 31.

[1] Geiger, A., *Proc. Roy. Soc.*, B, **107**, 368 (1930).
[2] Reiner, L., Moore, D. H., Lang, E. H., and Green, M., *J. Biol. Chem.*, **146**, 583 (1942).
[3] Munro, M. P., and Munro, F. L., *J. Biol. Chem.*, **150**, 427 (1943).
[4] Cabannes, R., and Serain, C., *C.R. Soc. Biol., Paris*, **149**, 7, 1193 (1955).
[5] Giri, K. V., and Pillai, N. C., *Nature*, **178**, 1057 (1956).
[6] Chernoff, A. I., *New Engl. J. Med.*, **253**, 322 (1955).

Some Nutritional Requirements of *Colletotrichum coffeanum* Noack

IN the course of the studies on the physiology of plant pathogenic fungi causing diseases in tropical crops of Mexico, several strains of *Colletotrichum coffeanum* Noack were isolated from coffee plants (*Coffea arabica*) showing typical signs of leaf spotting and die-back. Very little is known about the nutritional requirements of this fungus, although observations concerning the taxonomy and pathogenicity have been reported from time to time[1]. However, some previous reports have made reference to various aspects of the physiological behaviour of the conidial stage of *Glomerella cingulata*[2], which is identified as *C. coffeanum*[3]. The purpose of this communication is to summarize our observations on one of the isolated strains.

A stock culture of *C. coffeanum* (*CF*–2) was maintained by serial transfers on potato–dextrose–agar slants. In all the experiments the fungus was grown in submerged culture using rotary shakers at 25° C. for 72 hr. The extent of growth was determined by weighing the previously washed and dried mycelium. Preliminary experiments revealed that the organism could grow in a wide range of *p*H values, from 2 to 9·5; 3·8 being the optimum. A basal medium was selected consisting of KH_2PO_4, 1·00 gm.; K_2HPO_4, 1·30 gm.; KCl, 0·50 gm.; $MgSO_4.7H_2O$, 0·50 gm.; $(NH_4)_2SO_4$, 2·0 gm.; distilled water, 1 litre and the *p*H adjusted to 3·8. All the chemicals were analytical grade. For the experiments on the effect of trace elements, a modified Pfeffer's solution was used containing, KH_2PO_4, 5 gm.; $MgSO_4.7H_2O$, 2·5 gm.; $(NH_4)_2SO_4$, 10 gm.; sucrose, 50 gm.; triple glass-distilled water, 1 litre. Most of the cultures were carried out in 125-ml. Erlenmeyer flasks containing 25 ml. of culture medium; however, for the experiments with trace elements it was found that 125-ml. polyethylene bottles could replace the 'Pyrex' glass flasks favourably, giving 2–5 times more efficiency in the growth response. As inoculum 2·5–3·0 millions of washed conidia obtained from submerged culture were added to each flask or bottle.

No significant stimulation was observed when the following vitamins at two concentrations (0·2 μgm. per ml. and 4·0 μgm. per ml.) were added separately to the basal medium: riboflavin, thiamine, pyridoxine, inositol, nicotinic acid, calcium pantothenate, *p*-aminobenzoic acid and folic acid. Biotin at the same concentrations gave slight stimulation. The mixtures of all vitamins did not significantly stimulate growth. These results indicated that *C. coffeanum* is self-sufficient so far as vitamins is concerned.

The utilization of different nitrogen sources was studied by adding the nitrogen compounds at a concentration of 0·5 gm. of nitrogen per litre of culture medium. The results (Fig. 1*a*) indicated that the fungus was able to assimilate nitrogen from both organic and inorganic sources. Among the inorganic sources, ammonium sulphate produced maximum growth; among the organic sources of nitrogen, DL-alanine was the best individual source, and a mixture of all the amino-acids tested supported maximum growth. Casein hydrolysates and some kind of peptones were satisfactory sources, whereas neopeptone (Difco) was a poor source of nitrogen.

Several carbon sources including carbohydrates, sugar alcohols and organic acids were added to the basal medium at a concentration corresponding to 8 gm. carbon per litre of medium. The results (Fig. 1*b*) indicated that D-fructose induced maximum growth; sucrose, maltose, D-galactose and D-glucose were fairly good carbon sources and, as a rule, sugar alcohols and organic acids did not support growth. When ammonium sulphate was replaced by DL-alanine as the nitrogen source, D-glucose appeared to be the best carbon source. The effect of trace elements was studied on a modified Pfeffer's solution purified by the Steinberg's technique[4] and dispensed in polyethylene bottles. The addition of iron and/or zinc ions at a final concentration of 0·2 μgm. per ml. favoured the growth of *C. coffeanum* strikingly (Fig. 1*c*). Combinations of iron, zinc and manganese ions gave the maximum growth response. Under the conditions of the experiments the addition of copper ions antagonized the favourable effect of iron ions.

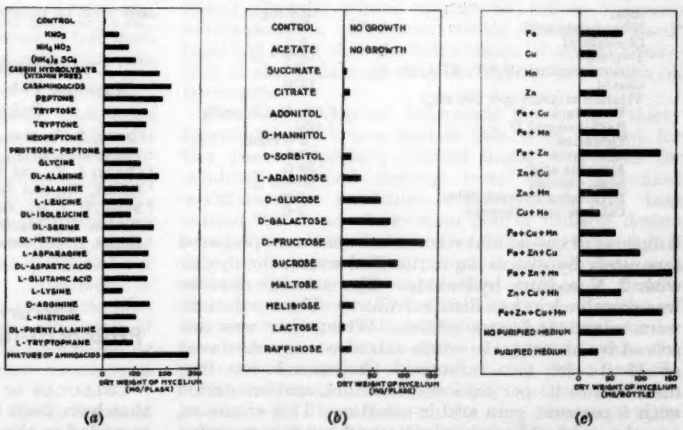


Fig. 1. (*a*) Growth of *C. coffeanum* on a basal medium containing different nitrogen sources; (*b*) growth of *C. coffeanum* on a basal medium containing different carbon sources; (*c*) effect of trace elements on the growth of *C. coffeanum*

Detailed accounts of the experiments will be reported elsewhere.

S. Bartniski-García
C. Casas-Campillo

Laboratorio de Microbiología,
Escuela Nacional de Ciencias Biológicas,
Instituto Politécnico Nacional,
México, D.F.

[1] Hastings de Gutierrez, L., *Turrialba* (Costa Rica), **4**, 115 (1954). Henrard, P., *Agricultura* (Louvain), **5**, 39 (1957). Rayner, R. W., *East African Agric. J.*, **17**, 130 (1952).
[2] Lin, Ch. K., *Amer. J. Bot.*, **32**, 296 (1945). Sussman, A. S., Coughey, P., and Strain, J. C., *Amer. J. Bot.*, **42**, 810 (1955).
[3] Small, W., *Kew Bull.*, **2**, 57 (1921).
[4] Steinberg, R. A., *Amer. J. Bot.*, **6**, 330 (1919).

Nutritional Requirements of the Common Housefly, *Musca domestica vicina* Macq

The housefly *Musca domestica vicina* Macq., which has been reared in our laboratory for four years with a modified NAIDM medium[1], was used in these experiments. Eggs were collected from oviposition dishes, washed and sterilized. The most satisfactory sterilizing solution and technique were as follows. Modified Vanderzant's solution[2] was prepared, with the omission of sodium glycocholate and less mercuric chloride (0·15 gm. instead of 0·25 gm.). Instead of a single treatment, the eggs were submerged in the solution twice, each for 10 min., with an interval of 1 hr. between the two treatments. After each treatment, the eggs were rinsed with bacteriologically sterile water. Plating on common bacteriological medium revealed that the percentage of contamination was less than 10 per cent and the percentage of eggs hatched was more than 50 per cent[3].

The sterilized eggs were transferred to cotton-stoppered vials with sterile media, one egg to each vial. Observations on the growth and development of the larvæ were made at frequent intervals and growth-rates recorded. Vials contaminated with micro-organisms were discarded.

The composition of the medium was based on the medium for *Hylemyia antiqua*[4] and *Calliphora erythrocephala*[5], since it is reasonable to assume that the nutritional requirements of these flies might be similar. The ingredients of the medium per ml. were as follows:

Agar	20·0 mgm.
Ribonucleic acid	1·0 ,,
Cholesterol	0·1 ,,
Dextrose	15·0 ,,
Mineral mixture (U.S.P. XIII, No. 2)	2·0 ,,
Casein	11·0 ,,
Vitamin mixture (per 100 ml.)	
Vitamin A	5,000 U.S.P. units
Rachitosterol D	1,000 ,, ,,
Thiamine	2·0 mgm.
Riboflavin	2·5 ,,
Ascorbic acid	30·0 ,,
Niacin amide	20·0 ,,
Pyridoxine hydrochloride	0·2 ,,
Calcium pantothenate	1·0 ,,

Solutions of casein and ribonucleic acid were prepared separately by dissolving in distilled water, the former with 2 *N* sodium hydroxide. The mineral mixture was dissolved in hot distilled water. These solutions were mixed and agar added. When agar was dissolved by heating, the whole mixture was autoclaved at 15 lb. for two minutes. Cholesterol was first dissolved in 95 per cent ethyl alcohol, and emulsified with 5 per cent gum arabic solution. This emulsion, together with dextrose solution and vitamin mixture, were then added. The final mixture was adjusted to *p*H 6·4 with 2 *N* sodium hydroxide. 2 ml. of this mixture was put in each food vial, a cotton stopper was used, and the vials were again sterilized at 15 lb. for 15 min.

Larvæ developed normally in the basic medium except that the larval period was somewhat prolonged and the pupal weight decreased (Table 1). This was probably due to the deficiency of glycine and cystine in casein. Further retardation of growth-rates resulted when ribonucleic acid or vitamins A, C and D were omitted. Non-sterile eggs on the same basic medium showed no advantages, suggesting that bacteria or other micro-organisms were not necessary symbionts for the housefly larvæ, at least when all other nutritional requirements were fulfilled.

Table 1. Growth-Rates and Pupal Weight of Houseflies Reared on Basic Medium and Modified Medium

Medium	Larval period			Pupal period (days)	Pupal weight (mgm.)
	First instar (days)	Second instar (days)	Third instar (days)		
Basic medium	1	1	3–6*	3–5	12–15
Basic medium less ribonucleic acid	3	3	3–4	4	11–15
Basic medium less vitamins A, C and D	1–3	1–2	3–5	3–8	12–15
Basic medium non-sterile eggs	1–2	1	5–11	3–4	11–13
Casein hydrochloride hydrolysate	1–3	3–5	—	—	
Casein pancreatin hydrolysate	1–2	2–4	2–4	6	11–12
Ten amino-acids	3–6	3	7	4	11
Modified NAIDM	1	1	3–4	3	15–18

* One larva had an extremely long third instar of 10 days.

Preliminary experiments on the amino-acid requirements of the housefly larvæ had been studied by using different hydrolysates of casein. The casein hydrochloride hydrolysate failed to support growth due to the lack of tryptophan, while pancreatin hydrolysate of casein (tryptophan not destroyed during hydrolysis) will support normal growth. Preliminary studies by varying the contents of amino-acid mixture to substitute for casein had also revealed that the essential amino-acids were probably the same for other dipterous insects such as *Drosophila melanogaster*[6] and *Pseudosarcophaga affinis*[7]. They are arginine, lysine, leucine, *iso*leucine, tryptophan, histidine, phenylalanine, methionine, valine and threonine; but the rate of growth with only these ten acids was very much retarded. Further studies are now in progress.

J. T. Chang
M. Y. Wang

Department of Biology,
National Peking University,
Peking.

[1] Chang, J. T., *et al.*, *Acta Ent. Sinica* (in the press).
[2] Vanderzant, E. S., and Reiser, R., *J. Econ. Ent.*, **49**, 7 (1956).
[3] Chang, J. T., and Wang, M. Y., *Scientia*, **11**, 335 (1957).
[4] Friend, W., and Patton, R. L., *Canad. J. Zool.*, **34**, 152 (1956).
[5] Sedea, D. J. W., *Acta Physiol. et Pharm. Neerl.*, **3**, 262 (1954).
[6] Hinton, T., *Genetics*, **40**, 224 (1955).
[7] House, H. L., *Canad. J. Zool.*, **32**, 358 (1954).

Occurrence and Filterability of Protoplast-like Elements in Aged Bacterial Cultures

Cellular or subcellular units of certain bacteria that have been deprived of a rigid cell wall have been regarded in the recent literature as protoplasts. The conversion of rods into spherical protoplasts was observed mostly in media containing penicillin and

magnesium ions plus sucrose as stabilizers[1], although Jeynes[2] has reported their natural occurrence.

The present communication describes the spontaneous occurrence of units in aged *Escherichia coli* cultures[3] which conform to the description of artificially induced bacterial protoplasts.

The *E. coli* strain used was isolated from human urine in 1952 and originally showed a certain degree of spontaneous polymorphism. Cultures were aged in horse-meat broth kept at 37° C. or at room temperature for several months in paraffin-sealed flasks. In electron-microscope preparations of samples from aged cultures the complete disintegration of some bacterial cells into naked plasmatic material containing electron-dense spots was readily observed. The disintegration was preceded by swelling of the cell and rupture of the rigid cell wall. Collodion membrane filtrates of the supernatant fluid (obtained after centrifugation at 2,000 r.p.m., 15 min.) of aged cultures contained units capable of regeneration when placed in fresh medium. The size of the smallest units so far found to regenerate has been estimated as approximately 350 mμ in diameter. Certain details of the regeneration were studied by light- and electron-microscopy. An initial fusion or coalescence followed by cell wall formation was noted. The individual protoplastic units were never observed to undergo division prior to fusion. The addition of washed *Sarcina lutea* cells to the filtrate appeared to stimulate the regeneration of the subcellular units. Microscopic observations and centrifugation experiments (Table 1) showed that this enhancement involved the adsorption of the protoplastic particles to the surface of the *Sarcina* cells.

Table 1. Ratio of the Number of Cultures showing Growth of *E. coli* Cells to the Number of Tubes inoculated with Variously Treated Filtrates from Aged Cultures

Samples	Experiment No. 1	Experiment No. 2
Filtrate (*a*)	7/33	5/35
Sediment (*b*)	2/30	0/35
Sediment (*c*)	16/35	19/38

(*a*) Aged broth culture was filtered through a membrane with an average pore diameter of 600 mμ; the filtrate was adjusted to *p*H 5·8.

(*b*) The filtrate (*p*H 5·8) was centrifuged at 1,000 r.p.m. for 20 min., the sediment was resuspended in the original volume of fresh broth.

(*c*) Filtrate (*a*) (*p*H 5·8) was centrifuged at 2,000 r.p.m. for 20 min., following the addition of approximately 10^6 *Sarcina lutea* cells per ml.; the sediment was resuspended in the original volume of fresh broth.

0·2 ml. amounts of all the samples were distributed in broth tubes and incubated.

These results support the assumption that aged *E. coli* cultures can survive in the form of sub-units having no cell walls which, under adequate conditions, can regenerate into the vegetative form. Recent observations on ribonucleic and deoxyribonucleic acids as well as protein synthesis in protoplasts or in their subcellular fractions[4,5] give credence to the conception that they may retain the potential for reversion into the bacillary form. However, in relation to the number of protoplastic structures seen in the electron microscopic fields, the efficiency of regeneration obtainable so far must be considered as poor, even in samples filtered through membranes with large pore diameters.

The morphological resemblance of the *L* forms of *Proteus* to the protoplasts was recently pointed out by Pease[6]. The stimulatory effect of *Sarcina* or other bacterial cells on the growth of both protoplasts and pleuropneumonia organisms[7] suggests the existence of similar metabolic pathways.

J. Sinkovics*

State Institute of Hygiene,
Budapest.

* Present address: Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey.

[1] Lederberg, J., *Proc. U.S. Nat. Acad. Sci.*, **42**, 574 (1956).
[2] Jeynes, M. H., *Nature*, **180**, 867 (1957).
[3] Sinkovics, J., *Acta Microbiol. Acad. Sci. Hung.*, **4**, 61 (1957).
[4] Ezekiel, D. H., and Spiegelman, S., *Bact. Proc.*, 137 (1957).
[5] Hahn, F. E., and Schaechter, M., *Bact. Proc.*, 138 (1957).
[6] Pease, Phyllis, *J. Gen. Microbiol.*, **17**, 64 (1957).
[7] Morton, H. E., Smith, P. F., and Leberman, P. R., *Proc. Soc. Exp. Biol. Med.*, **72**, 328 (1949).

Sexual Behaviour and the Vaginal Cycle in the Cat

Difficulties have been encountered in studying the neural and endocrine mechanisms which underly the control of sexual behaviour in the mammal for two main reasons. First, conspicuous and easily observable patterns of stereotyped behaviour, which are uninfluenced both by learning processes and the conditioning effects of the experimental situation itself, are rather few among laboratory mammals. Innate reflex patterns of behaviour similar to those successfully utilized by ethologists in lower animals, that are stable and difficult to disturb, occur quite infrequently in higher forms. The sexual behaviour of the female cat is a conspicuous exception to this. Though Marshall has emphasized the virtual impossibility of determining the state of the sexual cycle in many species by external signs alone, the present study shows this is not the case in the cat. The second source of difficulty arises from the observation that some of the components of the total pattern of sexual behaviour may not be dependent upon the secretory activity of the gonads; this is most evident in the male, but is also quite apparent in the female of several mammalian forms. The female chimpanzee may accept the male at any time in her cycle (Yerkes and Elder[1]); similar behaviour has been observed in the female rat (Boling *et al.*[2]); and acceptance by both dioestrous and ovariectomized rabbits is well established[3,4]. Sexual receptivity and the associated postural responses cannot be regarded as depending solely upon ovarian hormones in these species. This makes for difficulties in the interpretation of behavioural changes which appear to follow hormone administration. However, earlier studies by Bard[5] have indicated that the full pattern of sexual behaviour of the female cat may be strictly dependent on hormones.

Patterns of sexual behaviour shown by thirty reproductively active female cats were studied for two years. Specially trained males were used for conducting timed mating tests under standard environmental conditions. These tests are best carried out in an observation pen of suitable design where the male is allowed to establish territory rights. After an initial training period, a suitable male will mount and attempt copulation with any female that is presented. The normal sexual displays of mature, cycling females running with the male under free colony conditions were compared with the patterns of response obtained during these formally conducted mating tests, enabling an assessment to be made of the effect of the test situation upon behaviour throughout the cycle. The responses to artificial sexual stimulation and vaginal probing were also investigated.

Table 1

Smear	Behaviour: An-œstrus	Pro-œstrus	Œstrus	Met-œstrus	
An-œstrus	72·13 131 68·87	−12·41 8 20·41	−37·10 2 39·10	−12·60 1 13·60	142
Pro-œstrus	−14·92 20 34·92	24·65 35 10·35	−2·83 17 19·83	−6·90 0 6·90	72
Œstrus	−37·26 3 40·26	−6·93 5 11·93	49·14 72 22·86	−4·95 3 7·95	83
Met-œstrus	−9·95 8 17·95	−5·32 0 5·32	−9·19 1 10·19	24·46 28 3·54	37
	162	48	92	32	334

Table 2

$O - E$

O

E

$\frac{(O-E)^2}{E}$ =	$\frac{(O-E)^2}{E}$ =
75·544	35·202
6·375	0·404
34·484	105·632
5·515	8·288
7·546	11·674
58·707	6·900
4·026	3·082
5·320	169·009
	χ^2 = 537·707

$n = (4 - 1)(4 - 1) = 9$
From Fisher and Yates $\chi^2 = 27{\cdot}877$ for $P = 0{\cdot}001$.

It has been found that the behaviour patterns of individual animals are remarkably constant and that a well-defined behaviour cycle exists in this species. The behavioural characteristics specific to each of the four stages of the cycle, the anœstrous, the pro-œstrous, œstrous and metœstrous responses, will be enumerated in detail elsewhere. They were determined by combining the data on the responses of females to both artificial stimulation and the coital activity of the male during nine hundred mating tests. With the behavioural criteria established, an investigation was made of the relationship between behaviour and the cytology of the vaginal smear. 340 mating tests were conducted with 15 mature females and a corresponding number of vaginal smears collected. The description of the vaginal cycle given by Scott and Lloyd Jacob[6] was used, and the smears were examined without prior knowledge of the behavioural state of the animals from which they were obtained.

Correlating these data by means of a contingency table (Tables 1 and 2) reveals a highly significant association ($P < 0{\cdot}001$) between the behavioural patterns and the appearances of the vaginal smear at every stage of the cycle. The characteristic pattern of œstrous behaviour and the associated state of sexual receptivity, though commonly first appearing when the smear is pro-œstrous, continues throughout the period of full vaginal œstrous. The appearance of the metœstrous leucocytic infiltration in the smear closely coincides with the development of the metœstrous refusal pattern. Of the 142 observations on behaviour where the smear was anœstrous only once did mating occur, and in this particular animal the smear had cornified 24 hr. later. It can be concluded that mating behaviour with an anœstrous vaginal smear is a very unusual occurrence in this form, and further, that mating behaviour is almost invariably associated with either a pro-œstrous or œstrous smear. It was repeatedly observed that unless the specific œstrous posture is adopted, mating cannot take place no matter how energetic the copulatory efforts of the male. Only behaviourally œstrous animals can be mated; falsely positive mating tests due to 'rape' by the male do not occur in this species. Approximately 300 mating tests were conducted with 8 spayed females for periods of up to six months after operation during which time no vestige of œstrous behaviour was seen. Mating never occurred in this group even in those animals spayed at the height of œstrus and tested upon recovery from the anæsthetic 24 hr. later.

The highly conspicuous stereotyped mating response of the cat provides a sharp behavioural end-point for quantitative study and places it, in this respect, in a special position among laboratory mammals. The complete dependence of œstrous behaviour upon the presence of the ovaries has been confirmed beyond doubt. The relationship established between behavioural patterns and the state of the vaginal smear, together with the technique for conducting reliable mating tests, considerably facilitates the further study of sexual behaviour in this form.

Richard P. Michael*

Department of Neuroendocrinology,
Institute of Psychiatry,
Maudsley Hospital, S.E.5,
and Department of Physiology,
Royal Free Hospital Medical School,
8 Hunter Street, W.C.1.

* Fellowship in Clinical Research, Medical Research Council.

[1] Yerkes, R. M., and Elder, J. H., Comp. Psychol. Monogr., **13**, 1 (1936).
[2] Boling *et al.*, *Anat. Rec.*, **80**, 155 (1941).
[3] Brambell, F. W. R., *Proc. Zool. Soc. Lond.*, **144**, 1 (1944).
[4] Harris, G. W., Ciba Colloquia on Endocrinology, **3**, 58 (1952).
[5] Bard, P., *Res. Pub. Soc. Nerv. and Ment. Dis.*, **20**, 551 (1940).
[6] Scott, P. P., and Lloyd Jacob, M., "Studies on Fertility", **7**, 123 (1955).

Diseases of the Intervertebral Disk of the Cat

Although the domestic cat is known to suffer occasionally from spondylitic lesions of the vertebræ[1,2] it is not supposed to be susceptible to diseases of the intervertebral disks themselves[3]. If any species of mammal can be shown to be free, or relatively free, from diseases of the disk it should be profitable to compare the functional anatomy of its vertebral column with that of mammals known to be susceptible. This might uncover anatomical factors influencing diseases of the disk in general, or protrusion of the disk (herniation of the nucleus pulposus) in particular. This line of reasoning has in fact already been attempted on the basis that the dog and man are much more susceptible than the cat[3,4].

In 100 adult cats we have found dorsal (posterior) protrusions in 26, a total of 91 disks being affected. Two types could be distinguished: in one the protruded mass perforated the annulus fibrosus, and in the other it caused a bulge without passing right through the annulus. The distribution of protrusions along the vertebral column is summarized in the histogram in Fig. 1.

In 50 of the cats, we investigated the incidence of macroscopic degenerations other than dorsal protrusions. Ventral (anterior) protrusions were found in eight cats, and a lateral protrusion was found in one. Calcification was seen in three cats and 'brown

degeneration' in 15. General disorganization of the disk, sometimes attaining almost complete destruction, was much the commonest form of degeneration, for it was found in 27 cats and involved 178 disks; the distribution along the vertebral column of this general degeneration is summarized by the graph in Fig. 1.

These findings show that the cat is by no means indifferent to diseases of the intervertebral disk. If our survey of the incidence of dorsal protrusions in the cat is compared with the two main corresponding surveys in the dog[3,5] it appears that the cat is in fact nearly as susceptible as the dog. Unfortunately this nullifies the attempt, referred to above, to detect anatomical factors behind protrusion of the disk. However, it is a consolation that it helps previous efforts[3], hitherto based only on the dog, to dispose of the idea[6] that man's susceptibility to disk protrusion is simply due to his upright posture.

Analysis of the distribution along the vertebral column of dorsal protrusions and of other degenerations has been employed as another way of detecting anatomical factors. Taken as a whole, the first nine thoracic disks (*T* 1–2 to *T* 9–10 inclusive) are relatively immune to dorsal protrusion in the cat (and in the dog), even though general degeneration is particularly common. The conventional explanation for this in the dog is that the conjugal ligaments (which cross over each of these nine disks, in order to join the heads of nine pairs of ribs) support the disks dorsally and so prevent protrusion[3,5]. However, this is unlikely to be the whole answer. In the cat the first conjugal ligament is usually of negligible thickness, and therefore could scarcely be the sole reason for the rarity of protrusions from the disk which it covers (*T* 1–2). A similar objection applies for the dog[7].

In the dog the highest incidence of both dorsal protrusions and general degeneration occurs in the last thoracic disks, between *T* 10–11 and *T* 13–*L* 1[3]. This region includes the point of maximum normal kyphosis and the supposed site of maximum mobility of the trunk; consequently curvature and mobility seem to have been suspected as possible factors controlling disk disease in this quadruped[3]. The point of maximum normal kyphosis and the supposed site of maximum mobility[8] in the trunk are much the same in the cat as in the dog. Yet in the cat the incidence of dorsal protrusion and general degeneration is low in this region. This seems to show that curvature and mobility may not be so important after all. However, there are reasons why this argument may be altogether too facile. First, although kyphosis may look similar in the dog and cat, the anatomical reasons for it may be different. At least two independent features can produce it, wedge-shaped disks and wedge-shaped vertebræ; these have not yet been assessed in mammals other than man. Secondly, mobility seems to have been inadequately studied[9]. When these and other fundamental data are available about the functional anatomy of the vertebral column of various mammals, analysis of the distribution of lesions of their disks may be a useful approach to the underlying causes of diseases of the intervertebral disk and especially of dorsal protrusion.

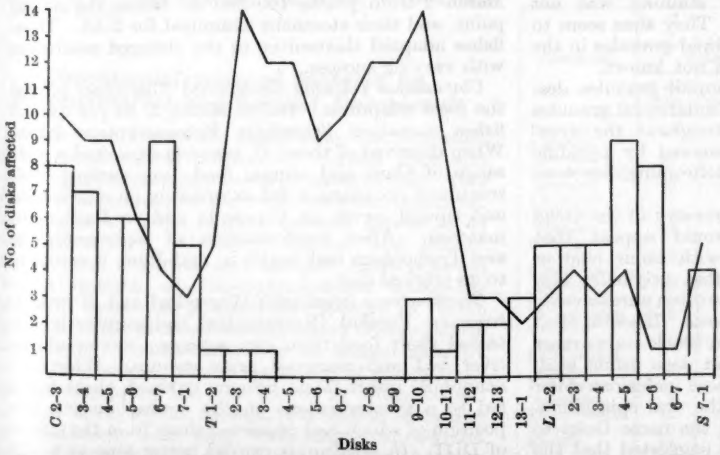


Fig. 1. Distribution of dorsal protrusions (histogram) and general degenerations (graph) of the intervertebral disks in the vertebral column

A. S. King
R. N. Smith

Department of Veterinary Anatomy,
University of Bristol.

[1] Glenney, W. C., *J. Amer. Vet. Med. Assoc.*, **129**, 61 (1956).
[2] Pommer, A. (quoted by Hutyra, F., Marek, J., and Manninger, R., "Special Pathology and Therapeutics of the Diseases of the Domestic Animals" (London, 1949).
[3] Hansen, H. J., *Acta Orthop. Scand.*, Supp. 11 (1952).
[4] Lindemann, K., and Kuhlendahl, H., "Die Erkrangungen der Wirbelsäule" (Stuttgart, 1953).
[5] Hoerlein, B. F., *Amer. J. Vet. Res.*, **14**, 260 (1953).
[6] Splithoff, C. A., *Amer. J. Surg.*, **71**, 19 (1946).
[7] King, A. S., *Vet. Rec.*, **68**, 939 (1956).
[8] Slijper, E. J., *Brit. Vet. J.*, **103**, 376 (1947).
[9] King, A. S., and Smith, R. N., *Brit. Vet. J.*, **111**, 135 (1955).

Sudanophil Inclusions in Ameloblasts, Odontoblasts and Cells of the Oral Epithelium

During studies of the lipid inclusions in cells of teeth and of the oral cavity of the rat, Baker's sudan black *B* method[1] was employed. The oral soft tissues were fixed in formalin–calcium solution, postchromed, embedded in gelatin, and cut on the freezing microtome. The enamel organ and pulp were dissected from the upper incisor tooth and similarly treated. Equally good results could be obtained from teeth if the entire tissue was decalcified with ethylenediamine tetraacetic acid (0·5 *M*) at *p*H 7·4, or with 10 per cent acetic acid, after fixation and before post-chroming. Beams and King[2] had investigated the Golgi bodies of dental tissues using Nassanow's method[3], and so this procedure was also used. Pyridine extraction of the tissues using Baker's method[4] was made in some cases. The results of the two methods agreed well on oral mucosa and that of the tongue.

Ameloblasts. Beams and King[2], using the Nassanow technique, described the changes in orientation of the osmic acid staining material in ameloblasts as these cells

approached maturity and functional activity. These results were confirmed by me using Nassanow's technique. But with the sudan black stain, deep blue or black granules were seen in the undifferentiated ameloblasts, scattered throughout the cytoplasm in an almost haphazard way. In the tall functional ameloblasts, the granules tended to be more on the enamel side of the nuclei (Fig. 1), but were still scattered throughout the cell. The cytoplasm on the enamel side of the nucleus took up more sudan black than that elsewhere. These granules were completely removed by pyridine if extraction was made before postchroming, but were little affected if extracted after postchroming. In contrast, the lipid granules of the oral mucosa were completely removed by pyridine whether extraction preceded or followed postchroming. Pyridine extraction likewise removed all osmiophil granules from ameloblasts and oral epithelium cells.

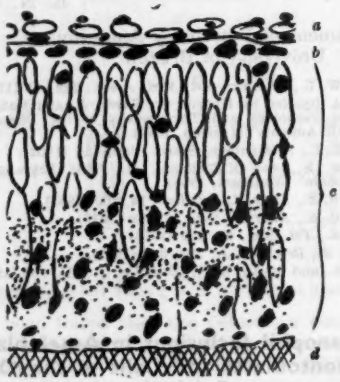


Fig. 1. Tall ameloblasts of the rat's upper incisor tooth stained by Baker's sudan black method. The sudanophil granules are black and the stippled area also stained with sudan black. × approx. 1,200.
a, Stratum intermedium; *b*, row of basal granules; *c*, tall ameloblasts; *d*, enamel

Basal granules. These are found only in the tall ameloblasts, at the stratum intermedium end of the cell. Beams and King[2] found them strongly osmiophilic, which I have confirmed. They also stained intensely with sudan black, with or without preliminary postchroming, and their staining was not affected by pyridine extraction. They thus seem to be chemically different from the lipid granules in the ameloblasts; but their nature is not known.

Odontoblasts. Unlike the osmiophil granules described by Beams and King[2], the sudanophil granules were distributed at random throughout the cytoplasm. These granules were removed by pyridine if extraction was done before postchroming, but were not soluble after postchroming.

Baker[5] has recently given a summary of the Golgi controversy, from which it would appear that inclusions in any cell, staining with osmic acid or silver, in a manner similar to that originally described by Golgi for nerve cells, have often unreservedly been called Golgi bodies or apparatus. It seems clear from the above results that sudan black stains more structures in the tooth cells than does osmic acid, and also that the properties of these inclusions differ in some respects from those of the oral epithelium. It is therefore unwise to append the name Golgi to them at all; in fact, Baker[5] has suggested that the name 'Golgi apparatus' should be abandoned.

Note added in proof. Since this communication was written, Symons[6] has reported findings with sudan black similar to those described above.

J. T. Irving
Joint Dental Research Unit of the C.S.I.R.O. and the University of the Witwatersrand, Johannesburg.
Oct. 17.

[1] Baker, J. R., *Quart. J. Micro. Sci.*, **90**, 293 (1949).
[2] Beams, H. W., and King, R. L., *Anat. Rec.*, **57**, 29 (1933).
[3] Nassanow, D. N., *Arch. mikr. Anat.*, **97**, 136 (1923).
[4] Baker, J. R., *Quart. J. Micro. Sci.*, **87**, 458 (1946).
[5] Baker, J. R., Symp. Soc. Exp. Biol., "Mitochondria and other Cytoplasmic Inclusions", 10 (1957).
[6] Symons, *J. Dent. Res.*, **36**, 805 (1957).

Effects of *Simulium* Control on Insectivorous Fishes

Extensive evidence from North America shows that DDT, at concentrations lethal to *Simulium* larvæ, need have no direct, harmful effect on fishes[1]. However, it may severely affect other aquatic insects[2–4], notably Ephemeroptera and Trichoptera[2,4,5], which are known to be important as food for many insectivorous fishes. It follows that the feeding habits of some fishes are likely to be modified considerably by insecticidal treatment, yet apparently this aspect of control has only once[6] received attention in the past. In view of the *Simulium* control measures envisaged in Africa, it is considered desirable to record some effects of DDT on the feeding habits of fishes in Uganda.

During March 2–May 4, 1956, the Victoria Nile at the Owen Falls, Jinja, was treated with DDT in order to eliminate larvæ of *Simulium damnosum* (Theobald) between Jinja and Mbulamuti. This dosing has been described elsewhere[6]. On several occasions before and during the treatment the sluice gates in the Owen Falls Dam were closed temporarily, resulting in marginal exposure of the river bed. Advantage was taken of this opportunity to sample the fauna and thus to assess the effects of the treatment.

In the Victoria Nile the DDT affected the invertebrate fauna differentially: some groups seemed unaffected, whereas almost all species of Ephemeroptera and Trichoptera were eliminated. Certain species of insectivorous fishes were collected before and after March 2 from points 100–500 m. below the dosing point, and their stomachs examined for food. These fishes adapted themselves to the changed conditions with varying success.

Clariallabes petricola Greenwood (Clariidae) proved the most adaptable. Before March 2, 83 per cent of fishes contained lithophilic Ephemeroptera larvæ. When deprived of these, *C. petricola* exploited a wide range of plant and animal food: on various post-treatment occasions it fed extensively on chironomid and tipulid larvæ, on Copepoda and on fresh plant material. After recolonization of Ephemeroptera and Trichoptera had begun in mid-June, it returned to its original diet.

Gnathonemus longibarbis Hilgendorf and *Mormyrus kannume* Forskal (Mormyridae) had previously obtained their food from two sources: stones of the river bed and marginal grass swamp. When the lithophilic insect fauna became reduced, these fishes fed to a greater extent on the swamp insects, the position of which had protected them from the effects of DDT. *G. longibarbis* proved better able to adapt itself in this way than *M. kannume*.

The most specialized feeding habits were encountered in *Mastacembelus victoriae* Boulenger (Mastacembelidae), of which 92 per cent had been feeding on lithophilic Ephemeroptera larvæ before March 2. For at least five weeks after the dosing, specimens were very scarce, and only 50 per cent (as against 94 per cent previously) of the fishes collected had food in the gut. Then the commonest items of food were small fishes and Hirudinea, which had seldom featured in earlier gut samples. It appears that *M. victoriae* either moved away from the area, or else was obliged to suffer starvation. Like *C. petricola* it gradually resumed its original feeding habits when repopulation of the lithophilic insects took place.

These observations provide an indication of responses to DDT to be expected in certain insectivorous fishes. In the present instance, effects of treatment were greatly mitigated by the rapid recolonization of the main food items, which occurred from unaffected breeding populations upstream from the dosing point. This mode of relief, however, must be regarded as exceptional, since most *Simulium* control in Africa is planned for highland streams where repopulation is unlikely to take place rapidly enough to be effective. Since many fishes in such habitats rely mainly on lithophilic insects for food, *Simulium* control there may cause the extinction of many little-known or undiscovered animals. It is felt, therefore, that a strong case exists for making collections and hydrobiological surveys before extensive control measures are put into force.

The work described here is to be published in full elsewhere.

PHILIP S. CORBET*

East African Fisheries Research Organization,
Jinja, Uganda.

* Present address: Virus Research Institute, Entebbe, Uganda.

[1] Jamnback, H., and Collins, D. L., N.Y. State Mus. Bull., No. 350, 1 (1955).
[2] Hoffman, C. H., Townes, H. K., Saller, R. I., and Swift, H. H., U.S. Dept. Agric. Bur. Ent. and Plant Quar. *E*-702, 1 (1946).
[3] Arnason, A. P., Brown, A. W. A., Bredeen, F. J. H., Hopewell, W. W., and Rempel, J. G., *Sci. Agric.*, 29, 527 (1949).
[4] Hoffman, C. H., and Merkel, E. P., *J. Econ. Ent.*, 41, 464 (1948).
[5] Adams, L., Hanavan, M., Hosley, N., and Johnston, D., *J. Wildlife Manag.*, 13, 245 (1949).
[6] Barnley, G. R., Trans. Tenth Int. Congress Ent., Montreal, 1956 (in the press).

Photosensitization of Bacteria by Furocoumarins and Related Compounds

IT has been known for many years that exposure to the simultaneous presence of various dyes and visible light results in the death of bacterial cells. This effect has been found to vary with different organisms, but in general, Gram-positive bacteria appear to be more sensitive[1,2]. Recently, Stainer and Cohen-Bazire[3], and Dworkin and Stanier[4] have reported that a mutant of *Rhodopseudomonas spheroides* devoid of carotenoid is killed on exposure to visible light, due to the photosensitizing action of endogenous chlorophyll. Photosensitization of human skin by topical application of another group of compounds, the furocoumarins, has been shown by Musajo *et al.*[5] to result in erythema, œdema, and vesicle formation on irradiation with long wave-length ultra-violet light. Fitzpatrick *et al.*[6] have demonstrated the same responses, as well as increased pigmentation, upon ingestion of the furocoumarin 8-methoxypsoralen. Photosensitization of bacteria by psoralen and related compounds is described in this communication.

The compounds were tested for activity by a paper-disk diffusion method on agar plates. The nutrient agar medium contained 0·3 per cent beef extract, 0·5 per cent peptone, 0·1 per cent yeast extract, 0·1 per cent dextrose, 0·05 per cent dipotassium hydrogen phosphate and 1·5 per cent agar. Whatman No. 2 filter paper disks, 6·1 mm. in diameter, were impregnated with 100 μgm. of the test compound. Six disks impregnated with different compounds were arranged uniformly on a freshly solidified 12-ml. agar layer in a 10-cm. Petri dish, with a dish impregnated with psoralen in the centre. A 6-ml. agar layer seeded with 0·02 ml. of an overnight broth culture of the test bacteria was carefully poured over the disks. After solidification of the second layer, the plates were incubated in the dark at 37° for 3 hr. before irradiation, to allow for diffusion of the test compound through the agar. Duplicate plates were left in the incubator to determine antibacterial activity. The test preparations, with the Petri dish covers removed, were irradiated 15 cm. from the pressed glass Wood's filter of a Model 70 Glo-Craft lamp (Switzer Bros., Inc., Cleveland, Ohio). The principal output of this lamp is the 3654-A. band of the mercury high pressure arc spectrum. After irradiation, the plates were reincubated in the dark at 37° overnight.

Single strains of *Bacillus subtilis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis* were tested against each compound at irradiation times of 20 and 40 min. Activity of the compounds was determined by measuring the diameter of bacteria-free zones surrounding the impregnated disks; the minimum measurable zone of inhibition was 6·1 mm., the diameter of the disk. The irradiation procedure itself had no effect on bacterial growth. Table 1 presents the results of 20-min. irradiation of those compounds found to be active. Irradiation for 40 min. resulted in only slightly larger zones. As noted in Table 1, partial, rather than complete, inhibition was observed with four compounds against some organ-

Table 1. INHIBITION PRODUCED BY ULTRA-VIOLET IRRADIATION IN THE PRESENCE OF FUROCOUMARINS AND RELATED COMPOUNDS

Compound	Inhibition zone (mm.)					
	Gram-positive			Gram-negative		
	B. subtilis	*S. aureus*	*S. faecalis*	*E. coli*	*P. vulgaris*	*P. aeruginosa*
Psoralen	19	21	20	17	25	9
5'Methylpsoralen	19	21	19	10	17	0
5',8-Dimethylpsoralen	20	21	22	0	14	0
4,5',8-Trimethylpsoralen	21	23	18	0	10	0
5-Methoxypsoralen	16	18	18	0	10	0
8-Methoxypsoralen	19	20	20	12*	15	0
5,8-Dimethoxypsoralen	12	11*	10*	0	0	0
Psoralen quinone	16	20	12	8*	0	0
4,5'Dimethylangelicin	15	14	11	0	0	0
5-Methoxyangelicin	8	9	0	0	0	0
Thiocoumarin	12	12	9	9	10	0
Visnagin	16	17	12	0	0	0
Khellin	14	12*	11*	0	0	0

* Partial inhibition.

isms. In these cases the zone boundaries were not distinct. On non-irradiated plates there were a few instances where anti-bacterial activity of the compounds shown in Table 1 was observed. The zone diameters of inhibition on such plates were as follows: psoralen with *B. subtilis*, 7 mm.; 8-methoxypsoralen with *B. subtilis*, 9 mm.; psoralen quinone with *B. subtilis*, 12 mm.; with *S. aureus*, 11 mm., with *S. faecalis* and *E. coli*, 7 mm.; thiocoumarin with *S. aureus*, 8 mm. Photosensitization was demonstrated by the appearance of much larger zones of inhibition on the irradiated plates, and this effect compared with that of 16 hr. or more dark contact with the diffusing compound, leaves no doubt about the role of light in the phenomenon. Indeed, most of the furocoumarins and related compounds showed activity only on the irradiated plates. As with dyes and visible light, Gram-positive organisms are more susceptible to photosensitization by these compounds.

The following furocoumarin derivatives were found to be inactive: 8-hydroxypsoralen, 5-geranyloxypsoralen, 2-thio-8-methoxypsoralen, 5-amino-8-methoxypsoralen, 5-nitro-8-methoxypsoralen, and 3,4-dihydro-8-methoxypsoralen. Of the furochromones, khellin and visnagin were active, but chellolglycoside and visamminol were not. The pyranocoumarins samidin, dihydrosamidin, and visnadin were also inactive. 3-Hydroxyflavone and six naturally occurring flavanoids gave no photosensitization; conversely, antibacterial activity on the unirradiated controls of three of the compounds was lost on irradiation. Other related compounds showing no activity were *o*-hydroxycinnamic acid, caffeic acid, cinnamic acid, 2-acetobenzofuran, phloridzin, 4-(*p*-methoxyphenyl)-3-buten-2-one, and twenty-two variously substituted coumarins. Coumarin was inactive at the usual 100 μgm. per disk; but very slight photosensitization did appear at 200 μgm. per disk. Eosin and methylene blue were inactive when tested in this system.

Eight of the thirteen active compounds reported here are found in various plant species. All the active compounds are weakly fluorescent except for psoralen quinone and thiocoumarin. However, since many of the inactive compounds are also fluorescent, this characteristic cannot *per se* confer photosensitization activity. Estimation of the relative activity of the various compounds would be hazardous, since the comparative rates of diffusion are unknown. However, their similar molecular weights and solubilities should result in similar diffusion rates.

The simultaneous presence of bacteria and compound is required for the effect since pre-irradiation of disks impregnated with 8-methoxypsoralen either alone or on the base agar layer resulted in no activity against *S. aureus* or *P. vulgaris*, no matter whether the seed layer was added immediately following irradiation or after three hours incubation. Irradiation of mixtures of 8-methoxypsoralen and bacteria in saline, followed by conventional plating techniques for surviving organisms, clearly shows that this photosensitization is bactericidal. Investigations are in progress on the kinetics of the lethal action and the effect of environmental factors.

We are indebted for some of the compounds used in this study to Dr. B. E. Christensen, Oregon State College, Corvallis, Ore.; Paul B. Elder Co., Bryan, Ohio; Givaudin-Delawanna Inc., New York; S. B. Pennick Co., New York; Sandoz Ltd., Basel, Switzerland; Sunkist Growers, Corona, Calif.; Trubek Laboratories, E. Rutherford, N.J.; U.S.D.A. Fruit and Vegetable Research Laboratories, Pasadena, Calif.; and Van Ameringen Haebler Inc., New York.

This investigation was supported in part by Public Health Service grant number *C*2837 (*PET*).

W. L. Fowlks
D. G. Griffith
Evelyn L. Oginsky

Departments of Dermatology and Bacteriology,
University of Oregon Medical School,
Portland 1, Oregon.

[1] T'ung, T., *Proc. Soc. Exp. Biol. and Med.*, **33**, 328 (1935).
[2] T'ung, T., *Chinese Med. J.*, Supp. **3**, 304 (1940).
[3] Stanier, R. Y., and Cohen-Bazire, G., "Microbial Ecology", Seventh Symp. Soc. Gen. Microbiol., 56 (1957).
[4] Dworkin, M., and Stanier, R. Y., *Bact. Prog.*, 131 (1957).
[5] Musajo, L., Rodighiero, G., and Caporale, G., *Bull. Soc. Chim. Biol.*, **36**, 1213 (1954).
[6] Fitzpatrick, T. B., Hopkins, C. E., Blickenstaff, M. S., and Swift, S., *J. Invest. Derm.*, 25, 187 (1955).

Cell Wall Composition in Bacterial and *L* Forms of *Proteus vulgaris*

α-ε-Diaminopimelic acid is known as an important constituent of the 'basic structure' of the bacterial cell wall[1–3]. As *L* forms seem to have a much less rigid cell wall than bacteria, the assumption that the *L* forms lack a normal wall became a main issue[4,5]. It was suggested that penicillin blocks some of the steps necessary for the condensation of the definitive wall. Recently, Weidel[6] has concluded from his experiments on phage enzyme that the main site of action of penicillin is the 'basic structure', consisting of α-ε-diaminopimelic acid, glutamic acid, alanine, glucosamine and muramic acid. Accordingly, this fraction should be missing in the *L* forms.

Comparative studies of the amino-acid composition of the bacterial form and the unstable and stable *L* form of *Proteus vulgaris* have shown[7] that the unstable form still contains α-ε-diaminopimelic acid, whereas it is absent in the stable form. In recent studies, Weibull found small amounts of α-ε-diaminopimelic acid also in a stable *L* form strain[8].

Attempts were made to isolate the 'basic structure' from the *L* form of *Proteus vulgaris*. The material and culture methods used were the same as those described earlier[9,10]. Most of the experiments were carried out on a stable (*A* strain) form cultivated on penicillin-free media for more than 200 passages without any sign of reversion to the bacterial form, and on an unstable (*B* strain) form, which had been transferred about 100 times on penicillin-containing media, without losing the ability to form normal rods when penicillin was omitted.

To isolate the wall material a recently described[11] simple method was applied. Bacterial or *L* forms were cultivated in a liquid medium and harvested by centrifuging for 20 min. at 15,000*g*. After washing the organisms twice in a 2 per cent sodium chloride solution they were suspended in 1 *N* sodium hydroxide and heated at 60° C. for 60 min. After diluting the sodium hydroxide to 0·5 *N* the undissolved material was centrifuged for 30 min. at 25,000*g* and the resulting gelatinous sediment was suspended again in 1 *N* sodium hydroxide. After heating at 60° C. for another hour the sediment was kept at 37° C. for 12 hr., then centrifuged again, washed with

Table 1. Amino-acid Content of Hydrolysates from the Bacterial and Unstable *L* Form of *Proteus vulgaris*, expressed in mol. (α-ε-Diaminopimelic acid (DAP) referred to as 1) or in per cent of all the Compounds mentioned in the Table

	Mol.			Total (per cent)		
	Bacterial form		*L* form Wall fraction	Bacterial form		*L* form Wall fraction
	Whole cells	Wall fraction		Whole cells	Wall fraction	
DAP	1·0	1·0	1·0	0·63	8·0	18·2
Glutamic acid	26·0	1·8	1·1	16·3	14·5	20·0
Alanine	23·0	3·3	2·0	14·5	25·5	36·0
Glucosamine	2·2	0·3	0·35	1·35	2·4	6·9
Muramic acid	?	0·2	0·2	?	1·6	3·4
Glycine	9·3	1·0	0·16	5·8	8·0	2·9
Serine	11·7	0·8	0·12	7·3	6·4	2·2
Lysine	20·0	1·2	0·16	12·6	9·7	2·9
Valine	17·2	0·70	0·05	10·2	5·6	0·9
Leucine	27·0	1·3	0·12	17·0	10·5	2·2
Aspartic acid	21·7	0·8	0·20	13·6	6·4	3·6

1 *N* sodium hydroxide and then three times with distilled water. Then the sediment was suspended in 6 *N* hydrochloric acid and boiled under reflux for 36 hr. The hydrolysate was tested for its amino-acid content by two-dimensional paper chromatography using two solvent systems—*iso*propanol (70), acetic acid (10), water (20); and picolin (78), ammonia (2), water (20) or picolin-solvent and water-saturated phenol. For the qualitative determination of α-ε-diaminopimelic acid the method of Ruhland *et al.*[12] was used.

The quantitative determination of the amino-acids was carried out according to Barrollier[13]. As shown in Table 1, α-ε-diaminopimelic acid predominates strongly in the wall fraction. Threonine, tyrosine, methionine, histidine, phenylalanine, proline and cystine (cysteine) are scarcely detectable in the wall fraction and are therefore not mentioned in Table 1. The composition of this cell wall fraction is probably very similar to the fraction, which Weidel[6] was able to split off *E. coli* using the phage enzyme. According to the density of the spots in his Fig. 2, there are also considerable amounts of lysine, glycine and valine (leucine is not shown) besides the main components (α-ε-diaminopimelic acid, glutamic acid and alanine).

When treating the stable *L* form (*A* strain) in the same way, the alkaline solution becomes entirely clear, and nothing can be centrifuged down. Sometimes very little of a white sediment could be obtained, but the hydrolysate showed a very complex composition without a trace of α-ε-diaminopimelic acid.

On the other hand, when unstable *L* forms (*B* strains) were used there was a slimy sediment as in the bacterial form. The hydrolysate showed a very pronounced pattern on the chromatogram, much more like the 'basic structure' of the cell wall of Gram-positive bacteria than that of the bacterial form. More than 80 per cent of the ninhydrin-positive substances are main components of the 'basic structure'.

Table 2. α-ε-Diaminopimelic Acid (DAP) Content of the Hydrolysate from Whole Cells of the Bacterial and *L* Form of *Proteus vulgaris*; Percentage Calculated on a Molar Basis

	Bacterial form			*L* form		
	Total nitrogen (μmol.)	DAP (μmol.)	DAP (per (cent)	Total nitrogen (μmol.)	DAP (μmol.)	DAP (per (cent)
log. phase	357	0·31	0·085	470	0·82	0·17
Stat. phase	350	0·39	0·113	380	1·47	0·38

Further investigations will be made to find out if the high percentage of glycine, serine, valine and other 'impurities' in the wall fraction from the bacterial form is due to incomplete extraction or if it indicates that in the normal wall a protein component is present which is missing in the unstable *L* forms.

Although the results in Table 1 show that the 'basic structure' of the wall is also present in the unstable *L* forms, it might be possible that the total amount is much less than in the normal rods. To answer this question, rods and *L* forms were harvested from growing and resting cultures (bacterial form 4 and 25 hr., respectively; *L* form 18 hr. and 4 days), washed with 2 per cent sodium chloride and hydrolysed. Aliquots from the filtered hydrolysate were subjected to total nitrogen determination and to 1-dimensional chromatography in *iso*propanol–acetic acid, which allowed the measurement of α-ε-diaminopimelic acid. The results in Table 2 illustrate that in growing cultures as well as in resting ones the α-ε-diaminopimelic acid content of the *L* forms is higher than that of the rods.

Thus, penicillin does not prevent the synthesis of the 'basic structure' of the cell wall, nor does it prevent its condensation to an alkaline-insoluble macromolecular structure. The reason for the fragility of the unstable *L* forms must be sought in another wall component. The fact that our stable *L* forms lack the basic structure completely is in good agreement with our earlier findings[7,9] and indicates again that we are dealing with different types of *L* forms.

We are indebted to Dr. R. E. Strange, Dr. K. McQuillen and Dr. C. Weibull for kindly supplying the muramic acid and the α-ε-diaminopimelic acid. This work has been sponsored by the Deutsche Forschungsgemeinschaft.

O. Kandler
A. Hund
C. Zehender

Botanical Institute,
University of Munich.

[1] Work, E., *Nature*, **179**, 841 (1957).
[2] Cummins, C. S., *Int. Rev. Cytol.*, **5**, 25 (1956).
[3] Cummins, C. S., and Harris, H., *J. Gen. Microbiol.*, **14**, 583 (1956).
[4] Park, J. T., and Strominger, J. L., *Science*, **125**, 99 (1957).
[5] Lederberg, J., *Proc. U.S. Nat. Acad. Sci.*, **42**, 574 (1956).
[6] Weidel, W., and Primosigh, J., *Zeitschr. Naturf.*, **12**, *b*, 422 (1957).
[7] Kandler, O., Zehender, C., and Hund, A., *Zeitschr. Naturf.* (in the press).
[8] Weibull, C., *Acta Path. Scand.* (in the press).
[9] Kandler, O., and Kandler, G., *Zeitschr. Naturf.*, **11**, *b*, 252 (1957).
[10] Kandler, O., Zehender, C., and Müller, J., *Arch. Mikrobiol.*, **24**, 219 (1956).
[11] Kandler, O., Hund, A., and Zehender, C., *Arch. Mikrobiol.* (in the press).
[12] Rhuland, L. E., Work, E., Deham, R. F., and Hoare, D. S., *J. Amer. Chem. Soc.*, **77**, 4844 (1955).
[13] Barrollier, J., *J. Naturw.*, **42**, 416 (1956).

Antigenicity of a Bacterial Deoxyribonucleic Acid

Since earlier evidence (refs. 1–3 and Medawar, P. B., personal communication) suggesting a serological reactivity for deoxyribonucleic acid (or the deoxyribonucleic acid moiety of a nucleoprotein) has been indirect or controversial, the problem has been

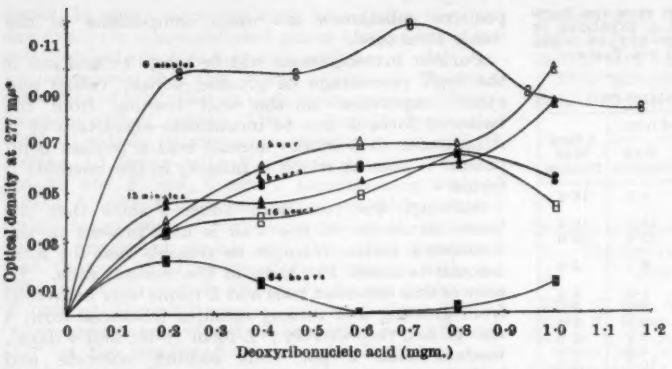


Fig. 1. Effect of deoxyribonuclease on quantitative precipitation. Antiserum, pooled rabbit anti-*Brucella abortus* – 19 *M* deoxyribonucleic acid; antigen, homologous deoxyribonucleic acid after treatment with Worthington deoxyribonuclease for various periods of time†.

* 277 mμ is the wave-length of maximum absorption for the dissolved precipitate in 0·25 *M* acetic acid.

† Action of deoxyribonuclease was terminated by addition of ethylenediamine tetraacetic acid to the reaction mixture.

re-investigated with the aid of a preparation rich in deoxyribonucleic acid with transforming activity[4,5], isolated from *B. abortus* cells by extraction with 0·5 per cent phenol in citrate–saline. The extracted material was 'deproteinized' by chloroform and amyl alcohol treatments[6], but the final material still contained, in addition to typical deoxyribonucleic acid[5], approximately 20 per cent protein that could not be dissociated by conventional procedures. Such preparations of deoxyribonucleic acid were used for immunizing rabbits, and the resulting antisera were tested first for precipitating antibodies against homologous deoxyribonucleic acid by the gel diffusion method[7].

Five bands of precipitation, indicating more than one antigen–antibody system, were detected. One of these bands yielded a strong Feulgen-positive reaction, suggesting the presence of deoxyribonucleic acid in the precipitate. The described reactions remained identical when the test antigen was treated either with detergent, 6 *M* sodium chloride, trypsin, trypsin plus chymotrypsin, or when the antisera were first absorbed with whole cells. However, even though deoxyribonuclease alone did not react with the antiserum, treatment of deoxyribonucleic acid with deoxyribonuclease (Worthington 1 × crystallized) resulted in a noticeable decrease in intensity of one of the bands and a simultaneous increase in another.

A more decisive indication of the involvement of deoxyribonucleic acid in these precipitin reactions was obtained by quantitative procedures[8] in which various amounts of antigen were mixed in test tubes with a constant amount of antiserum. The resulting precipitates were washed free of contaminating serum proteins and excess antigen, were dissolved in 0·25 *M* acetic acid, and were analysed spectrophotometrically at 277 mμ. Normal serum controls were also included in all tests. The number of maxima in the resulting quantitative precipitin curve (Fig. 1) again indicated the complexity of the material under study. However, what appears to be of greater significance are the changes occurring following treatment of the antigen with deoxyribonuclease for different periods of time. A significant decrease in the amount of precipitate occurred throughout most of the tested range following treatment with deoxyribonuclease for as little as 15 min. An analysis of the precipitate in the region of lowest antigen concentration, in terms of the ratio of absorptions at 260 and 277 mμ, revealed the presence of deoxyribonucleic acid which decreased with increasing time of treatment with deoxyribonuclease. This deoxyribonucleic acid could have been precipitated either because it contributed to the immunological specificity of the antigen, because it was firmly bound to the antigen, or because it co-precipitated with an unrelated antigen–antibody precipitate. The virtual abolition of antigenicity following prolonged enzymatic treatment, the absence of co-precipitation with known unrelated antigen–antibody precipitates, and the absence of any detectable proteolytic activity in the deoxyribonuclease used, favour the conclusion that deoxyribonucleic acid itself contributed to the specificity and is not merely an inert part of the antigens. This is further supported by the apparent interdependence of the specific precipitates formed as a function of the time of treatment with deoxyribonuclease (note vacillations in the quantity of precipitin formed and apparent shift of maxima to regions of higher antigen concentrations in Fig. 1). This may be due to the formation, during partial depolymerization of deoxyribonucleic acid, of antigenically active molecules with quantitatively different antigenic characteristics.

Additional support for the involvement of deoxyribonucleic acid in these antigen–antibody reactions has come from preliminary tests of cross-reactions between the described antisera and deoxyribonucleic acid from different sources. Deoxyribonucleic acid from both smooth and non-smooth *Brucella* cells gave the same patterns of precipitation in gel diffusion. In contrast, equal amounts of deoxyribonucleic acid isolated either from calf thymus (duponol-extracted) or salmon sperm (phenol-extracted) yielded much weaker reactions. However, exposure of the heterologous antigens to deoxyribonuclease resulted in a striking increase in their cross-reactivity, as evidenced by an increase in precipitation and the appearance of additional zones. Such effects would be difficult to explain on the basis of similarities in the protein moiety. Finally, preliminary evidence suggests a capability of these antisera to antagonize the selective effects of deoxyribonucleic acid breakdown products upon changes in population from non-smooth to smooth in growing *Brucella* cultures[4].

The present information fails to reveal whether the formation of specific antibodies requires protein bound to deoxyribonucleic acid or involves only the acid molecule. However, from a genetic, biological, and analytical point of view it would appear that the answer to this question may be less critical than the indication that deoxyribonucleic acid can contribute to the specificity of an antigen.

We wish to express our appreciation to Prof. M. Heidelberger for his interest, suggestions and stimulating discussions. This work was supported in part by grants from the U.S. Public Health Service

(*E*–1137) and the National Science Foundation (*G*–2184).

JOHN H. PHILLIPS*
WERNER BRAUN
OTTO J. PLESCIA

Institute of Microbiology,
Rutgers,
The State University,
New Brunswick, New Jersey.

* Waksman-Merck Postdoctoral Fellow. Present address: Department of Bacteriology, University of California, Berkeley, California.

[1] Lackman, D., Mudd, S., Sevag, M. G., Smolens, J., and Wiener, M., *J. Immunol.*, **40**, 1 (1941).
[2] Blix, U., Hand, C. N., and Stacey, M., *Brit. J. Exp. Path.*, **35**, 241 (1954).
[3] Masamune, H., and Tsuiki, S., *Tohoku J. Exp. Med.*, **61**, 171 (1955).
[4] Braun, W., and Whallon, J., *Proc. U.S. Nat. Acad. Sci.* **40**, 162 (1954).
[5] Braun, W., Whallon, J., and Phillips, J., *Nature*, **180**, 1356 (1957).
[6] Sevag, M., Lackman, D., and Smolens, J., *J. Biol. Chem.*, **124**, 425 (1938).
[7] Ouchterlony, O., *Arkiv. Kemi. Mineral. Geol.*, **26**, *B*, 1 (1948).
[8] Heidelberger, M., and Kendall, F., *J. Exp. Med.*, **50**, 809 (1929).

Relaxation of Snails before Fixation

NUMEROUS narcotizing agents have been used for relaxing snails, and among the more important of these employed recently are menthol and nembutal[1,2,4]. Menthol has been reported as giving variable results[4]; some snails become well extended, but others contract when this agent is used. This variation in results is observed especially when larger freshwater snails (shell height 15–25 mm.) such as *Lymnaea palustris* and *Physa gyrina* are placed in water to which menthol crystals have been added. On the other hand, smaller lymnæids such as *L. humilis* (maximum shell height, 12 mm.) relax well with menthol[3]. Nembutal gives good results with *Pomatiopsis lapidaria* and *P. cincinnatiensis*[4], but, like menthol, is unsatisfactory for the much larger *L. palustris*. This snail will contract slowly over a period of 4–5 min. when placed in 10 per cent formaldehyde even after being in rather high concentrations of nembutal (5 ml. stock veterinary nembutal in 150 ml. water at 4° C. for more than 40 hr.). Using this concentration of nembutal at room temperature results in contraction of *L. palustris* before it is properly narcotized, so that it is unsuitable for fixation. The same result occurs with snails in higher concentrations of nembutal (10 ml. in 150 ml. water) at 4° C.

However, by combining nembutal and menthol in the following way good results with *L. palustris* and *P. gyrina* are obtained. As many as 80 animals are placed in 150 ml. of tap water containing 1 ml. stock solution of veterinary nembutal (60 mgm. per ml.). The snails are left in the nembutal-treated water at room temperature for 1¼–1½ hr., and at the end of this time the soft parts are well extended. Then powdered menthol crystals are added (sufficient to cover the surface of the water), and the snails are refrigerated (4° C.) for 16–18 hr. in a capped jar. After this period of refrigeration, the snails can be fixed with the soft parts remaining in a well-extended position. When first placed in the fixative, slight movements of the tentacles may occur. During the period of relaxation in nembutal alone, the snails must be kept separated from one another and prevented from clinging to the sides of the container, otherwise interference with the extension of the foot may result. After the snails have been placed in the nembutal-treated water (before menthol is added), the time necessary for extension of the foot with cessation of movement is related to the size of the snail. *L. palustris* with a shell height of 6 mm. takes about 35 min., a 17 mm. snail about 55 min., and a 26 mm. snail about 65 min.

An interesting seasonal difference has been observed with *L. palustris* using this nembutal–menthol method of relaxation. Better results have been obtained with snails collected in the spring than with those collected in late summer and early autumn.

BRUCE M. MCCRAW

Division of Biology,
Ontario Veterinary College,
Guelph, Canada.
Nov. 14.

[1] Abdel-Malek, E. T., *J. Parasit.*, **37**, 321 (1951).
[2] Berry, E. G., Mus. Zool., Univ. Mich. Misc. Pap. No. 57 (1943).
[3] McCraw, B. M., *Can. J. Zool.* (in the press).
[4] van der Schalie, H., *Amer. Midl. Nat.*, **50**, 511 (1953).

Occurrence of a 'Rare' Earthworm in Montgomeryshire

A PRELIMINARY survey of the Lumbricidae in Montgomeryshire has revealed large numbers of *Eisenia veneta* (Rosa) var. *zebra* (Michaelsen) in compost heaps, manure, under decaying wood and under stones at Caersws.

Michaelsen's specimens were from the Caucasus, and up to 1947[1] the only British records were two specimens from Limerick[2] and one specimen (?) from Colwyn Bay, North Wales[3]. In 1954, two specimens were sent to the British Museum (Natural History) from a compost heap in Staffordshire.

Var. *zebra* occurs, in Caersws, together with the common lumbricids of compost heaps, *Dendrobaena subrubicunda* (Eisen) and *Eisenia foetida* (Savigny); but has not been found in similar habitats in any of the neighbouring villages.

K. SYLVIA RICHARDS

Department of Pharmacy and Biology,
College of Technology,
Birmingham.
Nov. 29.

[1] Cernosvitov, L., and Evans, A. C., "Synopses of the British Fauna". No. 6. Lumbricidae (Annelida), (Linn. Soc. London, 1947).
[2] Southern, R., *Proc. Roy. Irish Acad.*, **27b**, 119 (1909).
[3] Friend, H., *Northwestern Nat.*, **2**, 7 (1927).

Three Zoopagales from Brackish Water

THE Zoopagales are a small group of predacious fungi, at times placed in either the Mucorales or the Entomophthorales, but now more generally given ordinal rank. Most species prey on rhizopods (usually amoebæ); a few on eelworms, by adhesion to their rather sparse mycelium. Consequently they are most frequently found where these animals are active. Duddington[1] and Dixon[2] have recorded several species from compost heaps, decaying wood and moss cushions, and Juniper[3] from decaying animal dung, while Peach[4] restricted her searches to the aquatic habitats of pond and stream, which produced a remarkably rich flora.

So far no known recordings have resulted from saline habitats, but I have recently obtained three species from a salt-marsh in the Blackwater Estuary.

Samples were collected on three occasions during 1957 near Maldon, Essex; and consisted of decaying

debris and roots from tufts of the grass *Puccinellia maritima*, and swards of the alga *Enteromorpha*. Both regions were covered normally at high tides, with salinity readings of covering water of 24·6–32·4 (normal sea-water 35). Material was plated on rabbit dung agar. (2 per cent agar in water rendered a pale straw colour by addition of filtrate from stewed rabbit dung.)

Fungus *a* was readily identified as *Stylopage rhabdospora* Drechs.[5] by spore measurements, conidiophore structure and nature of the haustoria. This species is also common in terrestrial and fresh water habitats in Great Britain.

Fungus *b* may be provisionally described as a variety of *Stylopage rhynchospora* Drechs.[6] from which it differed mainly in smaller conidial size and the very slight and non-evacuated terminal beak to the conidium. It has not been recorded before from any other habitat.

Neither of these fungi produced zygospores or showed any evidence of sexuality, though from non-saline habitats these species do so very readily.

Fungus *c* has been recorded on two separate occasions from the same locality and cannot satisfactorily be placed with any of the described species of *Zoopage*. It may therefore be found necessary to create a new species to accommodate it, if warranted by further studies of this seemingly complex genus. This fungus is very persistent, and is characterized by the conidial chains of regularly only four conidia, each up to 60μ long and the stalked haustoria with short dichotomously branched lobes. Although under close observation for four months, no signs of sexuality have appeared.

The three fungi briefly described preyed on small amoebæ (unidentified) 10–18μ in diameter, and appeared 14–24 days after inoculation on several plates of each series. None showed sexuality, though most of the Zoopagales readily do so.

A full description of these fungi will be published later, when further work on similar habitats has been completed.

I wish to record my appreciation to Mr. C. Everard who collected the samples and determined the salinity readings, and Dr. C. L. Duddington for his interest and advice.

F. R. Jones

The Biological Laboratories,
The Polytechnic,
Regent Street,
London, W.1.

[1] Duddington, C. L., *Trans. Brit. Mycol. Soc.*, **34**, 322 (1951).
[2] Dixon, S. M., *Trans. Brit. Mycol. Soc.*, **35**, 144 (1952).
[3] Juniper, A. J., *Trans. Brit. Mycol. Soc.*, **40**, 346 (1957).
[4] Peach, M., *Trans. Brit. Mycol. Soc.*, **35**, 19 (1952).
[5] Drechsler, C., *Mycologia*, **28**, 363 (1936).
[6] Drechsler, C., *Mycologia*, **31**, 338 (1939).

Salmonellae in Uganda

This communication presents the preliminary results of an investigation into the carrier-rate of *Salmonellae* in zebu cattle arriving at the municipal abattoir of Kampala.

The cattle from which samples were collected had all been brought to Kampala from the Karamoja district, which occupies the north-eastern corner of the Protectorate and lies between latitude 1° 21′ and 4° 12′ N. and longitude 33° 20′ and 35° 0′ E. This district, which is administratively a closed area with relatively little contact with other territories, is the most primitive part of the Protectorate, and wild animals, including snakes and other reptiles, are present in considerable numbers.

Bacteriological examination was confined to fæces samples. The samples were collected by hand from the rectum a few hours after the animals had arrived at the abattoir. The samples were placed in a refrigerator within two hours of collection and cultured the following morning.

The method of isolation of *Salmonellae*, with a few minor modifications, was that used by Smith and Buxton[1] in the survey which they carried out in the United Kingdom. The types of *Salmonellae* isolated were identified by orthodox biochemical and serological methods, and the majority of the cultures were also sent to the Salmonella Reference Laboratory in England for confirmation.

Up to the present time, samples of fæces from 1,618 cattle have been examined and 33 samples, or 2·0 per cent, have been found to contain *Salmonellae*.

The species of *Salmonellae* encountered during the survey are as follows (the figures in brackets indicate the number of times each type was isolated): *wien* (1), *heidelburg* (1), *shubra* (1), *stanleyville* (2), *ituri* (1), *makiso* (2), *oranienburg* (2), *newport* (1), *zega* (1), *orion* (1), *chandans* (2), *aberdeen* (1), *mishmar-haemek* (1), *brazil* (1), *cerro* (1), *chicago* (1), *kibusi* (1), *adelaide* (1), *nairobi* (2), *berkeley* (1), new type 1 (1), new type 2 (1), new type 3 (4), new type 4 (1), new type 5 (1).

The large variety of *Salmonella* types isolated and the complete absence of *S. dublin* is in striking contrast to the results of similar surveys undertaken in the United Kingdom, where in the course of five surveys[1–5] 250, or 96 per cent, of 260 *Salmonellae* isolated were *S. dublin* and the remainder consisted of four other types only.

The finding by other workers of various *Salmonella* types in lizards[6,7], snakes[8] and tortoises[9] might suggest that some of the types found during the present investigation had originated from reptiles or game. What their significance is in cattle this communication makes no attempt to decide. However, some of the types isolated have been implicated in the cause of disease elsewhere, and since, according to definition[10], all the known types are pathogenic for warm-blood animals, including man, the finding of a variety of these organisms in 2 per cent of the cattle entering an abattoir must be regarded as a potential danger to public health.

During the investigation five new types of *Salmonellae* were encountered. Their antigenic structures are as follows:

New type 1	1, 4, 12, 27 : *z* : z_6
,, ,, 2	16 : *y* : *e,n,x*
,, ,, 3	40 : z_{41} : 1,2
,, ,, 4	1, 42 : *c* : z_6
,, ,, 5	28 : 1, *w* : z_{10}

Types 1, 2 and 3 have been named *S. entebbe*, *S. kikoma* and *S. karamoja* respectively. Types 4 and 5 are still being examined at the Salmonella Reference Laboratory, and when their complete antigenic structures have been confirmed it is proposed to name the former *S. kampala* and the latter *S. moroto*.

It is intended to extend the survey to cover cattle other than those for slaughter and also to compare the carrier-rates in healthy and sick animals.

I wish to thank Dr. Joan Taylor, of the Salmonella Reference Laboratory, Colindale, for examining most of the *Salmonella* isolated. My thanks are also due to the Medical Officer of Health, Kampala, for allowing me to collect samples at the abattoir.

E. McAnulty

Animal Health Research Centre,
P.O. Box 24,
Entebbe, Uganda,
British East Africa.

[1] Smith, H. Williams, and Buxton, A., *Brit. Med. J.*, **i**, 1478 (1951).
[2] Ritchie, J. M., and Clayton, N. M., *Mon. Bull. Minist. Hlth. Lab. Serv.*, **10**, 272 (1951).
[3] Report, *Mon. Bull. Minist. Hlth. Lab. Serv.*, **14**, 132 (1955).
[4] Murdoch, C. R., and Gordon, W. A. M., *Mon. Bull. Minist. Hlth. Lab. Serv.*, **12**, 72 (1953).
[5] Stewart, T., *Vet. Rec.*, **69**, 92 (1957).
[6] Mackey, J. P., *E. Afr. Med. J.*, **32**, 1 (1955).
[7] Collard, P., and Montefiore, D., *Nature*, **179**, 164 (1957).
[8] Hinshaw, W. R., and McNeil, E., *Amer. J. Vet. Res.*, **6**, 264 (1945).
[9] Boycott, J. A., Taylor, J., and Douglas, S. H., *J. Path. Bact.*, **65**, 401 (1953).
[10] Bergey, "Manual of Determinative Bacteriology", 492, 6th edit. (Baillière, Tindall and Cox, London, 1948).

An Inexpensive Easily Constructed Fish-marking Tag

A simple inexpensive fish tag, after the style of the hydrostatic tag devised by Einar Lee, has been constructed as follows (Fig. 1). Transparent vinyl plastic tubing (Crystal Vinyl tubing No. 6*A*, supplied by Portlant Plastics, Ltd.) $\frac{1}{8}$ in. int. diam. is cut in lengths of 2 in. One end of the tube is softened by heating near the edge of a very low Bunsen flame and then the walls are pressed together with large forceps. Dipping the forceps in cold water between each operation keeps them cold and thus increases the desired effect of welding the inner walls of the tube while minimizing distortion of the outer walls which cool more rapidly by contact with the cold forceps. Cooling the forceps also eliminates a tendency of the warm vinyl plastic to stick to them. A slip of white paper with the mark number and instructions in indian ink for the finder is next inserted and then the other end is closed. In one of the flattened ends a hole is made to take a 12-in. length of 0·65 mm. diam. monofilament nylon (sold for anglers as 27½ pounds or 12·5 kilos breaking strain). After insertion the nylon is knotted to form a loop. The cost of materials works out at less than 4*d.* a tag.

The tag is attached to the back of the fish, just anterior to the dorsal fin, in the case of pike and in front of the posterior dorsal fin in the case of pollack. A darning needle (which has the eye cut open on one side to hold the nylon) is used for drawing the nylon loop through the skin and the muscles, and a double looped knot is made to hold the tag in place. Darning needles are now used instead of medical suture needles which were originally used. The former are easier to insert and are considerably cheaper.

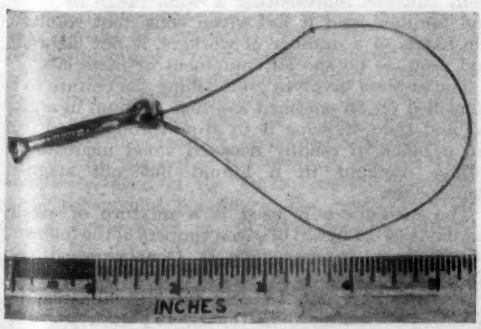


Fig. 1

We have used this tag in both freshwater (for pike) and sea water (for pollack). So far we have only had recoveries of pike; but these show that the tag is satisfactory. The wound made in the fish by tagging heals rapidly when this method is used and is completed in pike by the end of 48 hr. at water temperatures of about 10° C.

For freshwater work, at least, the tag has two advantages over the Einar Lee type; it is considerably less expensive and it is less conspicuous when submerged.

Fergus J. O'Rourke
P. Makings

Department of Zoology,
University College,
Cork.
Dec. 3.

Chromosome Number of *Marsilea*

The genus *Marsilea* has not previously been investigated by modern cytological methods, and we have only the early investigations of Strasburger[1] and de Litardiere[2] using techniques which experience has shown[3] to be unreliable when applied to ferns. It is therefore perhaps of importance to record a clear count of $n = 20$ (see Fig. 1) for a race of *Marsilea minuta* from northern India. We believe this to be

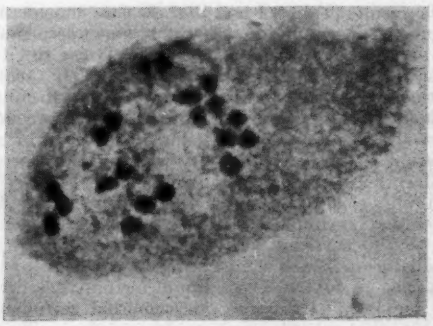


Fig. 1

a diploid and have found $2n = 40$ in squashes of root tips. Other cytotypes are, however, also present in this species, notably a sterile triploid with $2n = 60$, which reproduces vegetatively but which is otherwise very like the diploid and frequently intermixed with it. Further observations on this material will be published elsewhere.

P. N. Mehra
D. S. Loyal

Botany Department,
Panjab University,
Amritsar, India.

[1] Strasburger, E., *Flora*, **97**, 123 (1907).
[2] Litardiere, R. de, *La Cellule*, **31**, 255 (1921).
[3] Manton, I., "Problems of Cytology and Evolution in the Pteridophyta" (Cambridge Univ. Press, 1950).

Growth Responses of *Fusarium oxysporum* to Metabolites of Some Rhizospheric Microflora of Egyptian Cotton Varieties

During studies on the rhizospheric microflora of different cotton varieties grown in Egypt, *Fusarium* wilt-resistant Ashmouni cotton variety rhizosphere proved to be inhabited by the highest percentage of strains of *Bacillus subtilis*, while susceptible Giza 26 cotton variety rhizosphere contained the highest percentage of a strain of *B. megatherium*[1].

Preliminary screening tests in which each of the bacterial strains was streaked against *Fusarium oxysporum* on either Dox's or soil-extract agar media have proved that *B. subtilis* strains were highly antagonistic to the wilt-inducing pathogen; the former spread rapidly and overgrew *Fusarium* mycelium and encircled it completely. Hyphæ of *Fusarium* were lysed and malformed. On the other hand, *B. megatherium* was stimulatory to *Fusarium* mycelial growth. The latter overgrew *B. megatherium* and produced comparatively very abundant conidia with abnormally large size and high septation.

Growth responses of *Fusarium oxysporum*, which proved to be a virulent pathogen causing wilt of either Karnak or Giza 26 cotton variety seedlings, to metabolites of either *B. subtilis* or *B. megatherium* on Dox's liquid medium have been tested by the following two methods: (1) germinative potentialities of *Fusarium* microconidia in hanging-drop cultures of either *B. subtilis* or *B. megatherium* metabolites; (2) growth of *Fusarium* mycelium on differently treated metabolites of either *B. subtilis* or *B. megatherium*. Filtrates of the test organisms were obtained from cultures of two ages, that is, 5- and 10-days old and were either cold-sterilized[2] or autoclaved. Control solutions using either Dox's liquid or water were also used.

The results (Tables 1 and 2) show that considerable suppressed germination of *Fusarium* microconidia and mycelial growth took place in response to either 5- or 10-days old culture filtrates of *B. subtilis* whether unheated or autoclaved. Ageing of culture or application of heat to its filtrate did not appreciably affect the latent period and final percentage of germination or the mycelial dry-weight of *Fusarium*. On the other hand, on using filtrates of *B. megatherium* from either 5- or 10-days old cultures, the final percentage germination of *Fusarium* microconidia rose to 100 per cent. At the same time, *Fusarium* mycelial growth was significantly higher as compared with that on control Dox's liquid media. A stimulatory factor for *Fusarium* microconidial germination and mycelial growth seems to be present in filtrates of *B. megatherium* the potency of which is not affected by heating. On the other hand, ageing of culture results in an increased stimulative effect over *Fusarium* mycelial growth.

Table 1. Growth Responses of *Fusarium* Microconidia to Metabolite-Filtrates of *Bacillus subtilis* and *B. megatherium* at 30° C.

Experimental medium	Age of culture	Filtrate treatment	Germinative capacities	
			Latent period	Germination (per cent)
B. subtilis filtrate	5 days	unheated	5 hr.	40
		autoclaved	5 hr.	40
	10 days	unheated	5 hr.	48
		autoclaved	5 hr.	50
B. megatherium filtrate	5 days	unheated	5 hr.	100
		autoclaved	5 hr.	100
	10 days	unheated	5 hr.	100
		autoclaved	4 hr.	100
Pure Dox's	—	—	2 hr.	90
Water	—	—	3 hr.	80

Table 2. *Fusarium* Mycelial Dry Weight (in mgm.) After 10 Days at 30° C. in Response to Differently Treated Metabolic-Filtrates of *B. subtilis* and *B. megatherium*. Pure Dox's Liquid is Diluted with Water and Metabolite Filtrates with Pure Dox's Liquid*

Experimental medium	Dilution (per cent)	Filtrate from 5-day old cultures		Filtrate from 10-day old cultures	
		Unheated	Autoclaved	Unheated	Autoclaved
B. subtilis filtrate	100	33	35	29	30
	50	38	40	33	35
	25	45	48	38	41
B. megatherium filtrate	100	40	43	38	42
	50	73	77	80	85
	25	81	85	92	105

* Mycelial dry weight on 100 per cent pure Dox's liquid, 75 mgm.; on 75 per cent Dox's liquid, 61 mgm.; on 50 per cent Dox's liquid, 40 mgm.

Further work concerning the nature of the inhibitory anti-fungal metabolites of *B. subtilis* and the stimulatory factor of *B. megatherium* for *Fusarium* growth are still in progress. The former are probably of the nature of an antibiotic[3] while the latter seems to be a growth factor. Difference in the rhizosphere microflora of wilt-resistant and susceptible cotton varieties might explain why *Fusarium oxysporum* fails to invade Ashmouni cotton roots while its pathogenicity is established on susceptible Karnak and Giza 26 cotton varieties.

Thanks are due to Prof. A. H. Montasir for his unfailing help.

M. S. Naim*
A. M. Hussein

Faculty of Science,
A'in Shams University,
Abbassia, Cairo.

* Present address: Shambat Agricultural Institute, P.O. Box 71, Khartoum North.

[1] Naim, M. S., Mahoud, S. A. Z., and Hussein, A. M., *A'in Shams Sci. Bull.*, No. 1, 173 (1956).
Menon, K. P. V., *Ann. Bot.*, **48** (1934).
[2] Prescott, S. C., and Dunn, C., "Industrial Microbiology", 2nd Edn. (McGraw-Hill Co., 1949).

A Simple Technique for producing Fruit Bodies of Wood-destroying Basidiomycetes

In attempts to identify some of the many basidiomycete cultures isolated in our investigations of decay, we have tested a number of methods and have found that described here of the greatest value. It produces relatively large, identifiable fruit bodies from cultures representing a wide range of genera and families, and it has given consistent results in the hands of a number of workers, is not laborious, and requires no special equipment or materials.

The method involves establishing the culture to be identified on an enriched sawdust medium in a glass jar and then allowing it to grow through, and fruit on, a block of readily decayed wood incubated in diffuse daylight in a humid but not stagnant atmosphere.

The medium now used is a mixture of air-dry sawdusts (4–24 mesh) of seven timbers in the following proportions: *Pinus radiata* D. Don (sapwood), 25; *Eucalyptus obliqua* L'Herit. (sapwood), 25; *E. diversicolor* F. v. M., 10; *Acacia dealbata* Link., 10; *Nothofagus cunninghamii* Oerst., 10; *Rhizophora* sp. 10; *Ceratopetalum apetalum* D. Don, 10. (This

Fig. 1. Production of fruit bodies of wood-destroying basidiomycetes. 1, culture jar, with cover jar, after incubation at 25° C.; 2, culture jar showing fruit bodies of *Fomes gilvus* (Fr.) Lloyd on wood block

complex mixture, intended to ensure that fungi showing a strong host preference for fruiting in Nature will receive any accessory nutrients they require, is based on the general origins of our culture collection, and may be varied to reflect the origins of particular cultures.) The following nutrients are added to each 100 gm. of air-dry sawdust: 2·5 gm. maize meal; 1·5 gm. bone meal; 0·75 gm. potato starch; 0·2 gm. dried yeast; 0·5 gm. malt extract; 0·175 gm. casein hydrolysate; 0·0033 gm. thiamine hydrochloride. The first four are mixed in powder form with the dry sawdust; the last three are dissolved beforehand in the water used for moistening it. Sufficient water is added to saturate the sawdust particles without allowing free water to collect at the base of the culture jar, the amount required being determined for each batch of mixed sawdusts by draining saturated samples for 48 hr.

Enough moist nutrified sawdust is packed into a glass jar, 5¼ in. high and 2¼ in. wide, to come slightly above the mouth, which is then covered with an inverted wider jar (Fig. 1, 1) and the assembly autoclaved. An inoculum from a Petri-dish colony of the test fungus on malt agar is placed just below the surface of the sawdust and the jar incubated in darkness at 25° C. until a vigorous mycelium develops throughout the medium (Fig. 1, 1).

The inverted cover jar is then aseptically replaced by a sterile wooden block, 2¼ in. square by 1¾ in. high, with a ¾-in. deep well in the lower face to fit over the mouth of the jar, and a ¼ in. deep well in the upper face as a water reservoir (Fig. 1, 2). Blocks are usually either *Pinus radiata* sapwood or balsa, and are brought to a suitable moisture content for fungal growth (approximately 100 per cent for *P. radiata* and 300 per cent for balsa) before being autoclaved. It is essential that the block makes good contact with the top of the sawdust and fits tightly around the neck of the jar to prevent movement during subsequent handling. The block is covered with a sterile sheet of polyethylene film, gathered around the neck of the jar by a rubber band, to exclude contamination.

The culture jars are then incubated at laboratory temperatures (12–25° C.) in a shallow tank with a plate-glass lid, receiving abundant diffused light from the southern (polar) sky but no direct sunlight. Water in the bottom of the tank maintains a high humidity, and aeration is provided by bubbling compressed air through water in a flask and then into the tank. As soon as the fungus reaches the surface of the wood block, the polyethylene film is removed and the block is kept moist by adding water periodically to the well in its upper face. (Some contamination, usually *Penicillium* spp., may develop on the blocks, but has little adverse effect, though *Trichoderma viride* will often completely inhibit fruiting.) Jars are disturbed as little as possible, and if removed for inspection are replaced in the same position and orientation.

Fruit bodies are usually produced by 30–40 per cent of the culture jars included in each experiment, the time taken (after the jars are placed in the tanks) varying from 2 to 3 weeks, for *Schizophyllum commune* Fr., to 6 months or more, for *Fomes gilvus* (Fr.) Lloyd. They are usually of normal shape and coloration and of reasonably large size although some pileate species develop only a resupinate form. Fruit bodies may develop on any face of the blocks, but tend to be more frequent on the end grain and on the side facing the light; many develop from the lower face of the block. Satisfactory fruit bodies have now been obtained with species of *Polyporus*, *Coriolus*, *Trametes*, *Hexagona*, *Fomes*, *Ganoderma*, *Elfvingia*, *Poria*, *Dædalea*, *Merulius*, *Corticium*, *Peniophora*, *Coniophora*, *Stereum*, *Hydnum*, *Hymenochæte*, *Odontia*, *Lenzites*, *Lentinus*, *Paxillus*, *Schizophyllum* and *Hypholoma*; but it is not claimed that the technique described is suitable for all species of these genera, and research is continuing on further modifications and on other methods.

N. Tamblyn
E. W. B. Da Costa

Division of Forest Products,
Commonwealth Scientific and
Industrial Research Organization,
Melbourne.

Comparative Chlorophyll Content of the Two Generations of Bryophytes

The occurrence of chlorophyll in the sporophyte of Bryophytes has been noted by a number of investigators. Bold[1,2] has reviewed the older literature, and, by cytological studies on living material, has demonstrated chloroplasts and starch grains in the foot, seta and capsule of a number of species of both liverworts and mosses. In the liverwort, *Riella americana*, "as the sporange approaches maturity, its color is much more deeply green than is that of the gametophyte"[3]. Douin[4] has examined the pigments of gametophytes of forty species of mosses belonging to twenty-eight genera, finding that all of them contain chlorophylls *a* and *b*, as in higher plants. In a comparative study of the sporophyte and gametophyte of five species of mosses, no qualitative differences in chlorophylls *a* and *b* or carotenoids were found[5].

It is the purpose of this communication to present quantitative results concerning the chlorophyll content of sporophytes and gametophytes of *Anthoceros carolinianus* Michx., *Polytrichum commune* L., and *Dicranum scoparium* (L.) Hedw. After macroscopic separation of the fresh plant material into gametophytic and sporophytic portions, the samples were weighed. Extraction of the pigments and spectrophotometric determination of the quantities of

Table 1

Species and generation	Chloro-phyll *a*	Chloro-phyll *b*	Total	Sporophyte/ gametophyte ratio
Anthoceros carolinianus				
Gametophyte	0·31	0·18	0·49	
Sporophyte	0·72	0·43	1·15	2·35
Polytrichum commune				
Gametophyte	0·07	0·05	0·12	
Sporophyte	0·19	0·10	0·29	2·41
Dicranum scoparium				
Gametophyte	0·13	0·09	0·22	
Sporophyte	0·21	0·15	0·36	1·64

chlorophylls present were performed in accordance with Official Methods of Analysis of the Association of Official Agricultural Chemists[6]. In Table 1, the chlorophyll concentrations found are expressed as mgm./gm. fresh weight.

While the much smaller sporophytes contain less total chlorophylls than the gametophytes, when comparisons are made on the basis of equal fresh weights, the chlorophyll concentration in the sporophytes exceeds that in the gametophytes by a factor of 1·64–2·41. These findings have an obvious bearing on the question of the nutritional relationship between the two generations.

Frederick T. Wolf

Department of Biology,
Vanderbilt University,
Nashville, Tennessee.

[1] Bold, H. C., *Amer. J. Bot.*, **25**, 551 (1938).
[2] Bold, H. C., *Amer. J. Bot.*, **27**, 318 (1940).
[3] Studhalter, R. A., *Ann. Bryol.*, **11**, 153 (1938).
[4] Douin, R., *C.R. Acad. Sci., Paris*, **243**, 1051 (1956).
[5] Freeland, R. O., *Plant Physiol.*, **32**, 64 (1957).
[6] "Official Methods of Analysis", 112, 7th edit. (Assoc. of Official Agricultural Chemists, 1950).

Propagation of Cacao in Plastic Bags

The basic physiological requirements for the vegetative propagation of cacao (*Theobroma cacao*) have been broadly worked out by Evans (1951) and may be summarized in that semi-hardwood cuttings in an atmosphere near 100 per cent relative humidity, at a reduced light intensity (15–20 per cent of the incident sunlight), given adequate aeration at the base of the cutting, will produce roots in the order of 70–90 per cent rooting efficiency.

A comparison of the costs of commercial propagation in Trinidad has been made by Murray and Bridge[1], and McKelvie[2] has shown that cacao may be propagated at greatly reduced cost, on a commercial scale, using sawdust-cored baskets[3] containing cacao cuttings, covered by polyethylene sheets. This method adequately dispenses with the expensive installation of commercial propagating bins, and considerably reduces water consumption and labour costs. Another method, making use of the properties of polyethylene, which restricts water-vapour diffusion but permits some gaseous diffusion, is being developed here. Cuttings are rooted entirely in plastic bags, transported to the field in the same fashion and afterwards established in the field.

The base of a semi-hardwood cacao cutting bearing 4–6 trimmed leaves is dipped in hormone solution. The base of the cutting is first placed in wet, leached sawdust laid in coconut fibre. The fibre is then folded around the base of the cutting and held in place with an elastic band. The final size of the sawdust and fibre 'ball' is about the size of a half-clenched fist. The cutting is inserted into a plastic bag (150 gauge, 20 in. × 11 in.), water (150–200 ml.) added and the neck closed with string. The bag is suspended under a shade of domestic cloth, and no further attention is required until root formation has occurred. The roots emerge through the fibre and may be readily seen through the plastic. The light-intensity, calculated by means of an actinograph, is approximately 7 per cent of the incident sunlight, but is not critical over wide limits providing the light is diffused.

The cutting is transported to the field in the bag and planted under heavy shade with the roots still enclosed in the fibre. The surplus water from the plastic bag is tipped over the plant and the bag is inverted afterwards over the cutting with the mouth of the bag lying loosely upon the soil surface. Two banana leaves, which provide local shade, are arranged in the form of an inverted V over the cutting, with the petioles firmly pressed into the soil. Natural drying-out of the banana leaves permits progressively more light to fall on the cutting. After 4–5 days, the bag is removed and re-used for further propagation. The purpose of the inverted bag is to reduce transpiration and replaces the conventional hardening procedure. The probable reason why success is achieved by this method is that the roots of the cutting are intimately interwoven with the wet fibre and sawdust, which retains moisture for a considerable time during the critical period when the roots are becoming established in the soil.

The rooting percentage in the bag is of the order of 80–90, depending upon the clone, and establishment in the field about 60–70 per cent of the cuttings set in the bags. Plants of the more vigorous clones are ready for planting 22 days from setting.

The advantages of the method are many. The plastic bag costs 2½*d.* and may be used a number of times without deterioration. Labour charges and initial installation are negligible when compared with conventional methods. Plants are introduced into the field more quickly and are very easily transported by hanging the bags from wires in a vehicle. Furthermore, drying-out, which is frequently observed with plants in baskets transported over long distances, does not occur. The combined weight of a plant and basket is between 3½ and 4 lb. as compared with 1 lb. for a plant in a plastic bag. One man may carry up to 20 plants in bags without difficulty, whereas he is restricted to two basketed plants owing to the weight and shape. This means that inaccessible bush areas may be more readily planted with clonal cacao. Skilled labour is not required at any stage in the process, and normal care is all that is required at planting.

Further work is being conducted on the storage of plants in bags containing a nutrient solution, and a full account will be published elsewhere.

Thanks are due to Mr. G. W. Smith for the measurements of light-intensity using the actinograph.

R. Nichols

Regional Research Centre,
Imperial College of Tropical Agriculture,
Trinidad.

[1] Murray, D. B., and Bridge, C. J. R., "A Report on Cacao Research", 41 (Imp. Coll. Trop. Agric., Trinidad, 1957).
[2] McKelvie, A. D., *Trop. Agric.*, **34**, 260 (1957).
[3] Murray, D. B., "A Report on Cacao Research", 53 (Imp. Coll. Trop. Agric., Trinidad, 1953).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, February 24

BRITISH SOCIETY FOR THE HISTORY OF SCIENCE (in the Council Room of the Science Museum, Exhibition Road, South Kensington, London, S.W.7), at 5.30 p.m.—Dr. A. Armitage and Dr. N. H. de V. Heathcote: "The First International Polar Year, 1882–83—Forerunner of the International Geophysical Year".

INSTITUTION OF ELECTRICAL ENGINEERS, RADIO AND TELECOMMUNICATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Informal evening on "Stereophonic Recording on Gramophone Discs". Talk by Mr. H. A. M. Clark.

UNIVERSITY OF LONDON (in the Chemistry Lecture Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. E. Heilbronner (Swiss Federal Institute of Technology, Zürich): "Some Studies on Non-Benzenoid Aromatic Compounds".*

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Mr. Eric Shipton: "The Imperial College Karakoram Expedition".

Tuesday, February 25

UNIVERSITY COLLEGE, LONDON (in the Physiology Theatre, Gower Street, London, W.C.1), at 5.15 p.m.—Dr. C. A. G. Wiersma (California Institute of Technology): "Integration in Single Units of the Crayfish Central Nervous System".*

INSTITUTION OF CIVIL ENGINEERS (at Great George Street, London, S.W.1), at 5.30 p.m.—Mr. L. R. Creasy: "Prestressed-concrete Cylindrical Tanks".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Prof. R. King: "Energy". (Further lectures on February 26 and March 4 and 5. Lectures for Sixth Form Boys and Girls from Schools in London and the Home Counties.)

SOCIETY OF CHEMICAL INDUSTRY, LONDON SECTION, and PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. G. Dring: "A Plastics Jubilee: 1907–1957" (Jubilee Memorial Lecture).

Wednesday, February 26

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Dr. P. W. Brian: "Gibberellic Acid—a New Plant Hormone Controlling Growth and Flowering" (The Fernhurst Lecture).

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Annual General Meeting. 5 p.m.—Sir Hugh Linstead, O.B.E., M.P.: "Science and Politics" (Bernard Dyer Memorial Lecture).

INSTITUTE OF FUEL (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Dr. J. A. Gray: "Boiler Water Treatment—a formula for the Control of Sludge and Scale in Internal (Carbonate) Treatment"; Mr. E. F. Thurston and Mr. L. Furnival: "Boiler Water Treatment—a Formula for the Control of Sludge and Scale in Internal Carbonate Treatment: Experiments in a Laboratory Boiler".*

ROYAL AERONAUTICAL SOCIETY (at Church House, Westminster, London, S.W.1), at 6 p.m.—Sir George R. Edwards—Presidential Address.

BRITISH INSTITUTION OF RADIO ENGINEERS (in the Lecture Theatre of the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6.30 p.m.—Mr. C. Powell and Mr. D. A. Hendley: "DECTRA—a Long-range Radio-navigational Aid".

WOMEN'S ENGINEERING SOCIETY, LONDON BRANCH (at the Central Electricity Authority Headquarters, Winsley Street, London, W.1), at 6.45 p.m.—Mr. Geoffrey Denton: "Three Case Studies in Automation".

Thursday, February 27

INSTITUTION OF THE RUBBER INDUSTRY; and the PLASTICS INSTITUTE (at the Institution of Civil Engineers, Great George Street, Westminster, London, S.W.1), at 9.45 a.m.—Joint Conference on "Recent Advances in Polymer Technology".

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. S. Cotson, Mr. S. J. Holt, Mr. D. G. O'Sullivan, Mr. P. W. Sadler and Mr. R. F. J. Withers: "Studies in Enzyme Cytochemistry".

INSTITUTION OF ELECTRONICS (in the Assembly Hall, University of London Institute of Education, Malet Street, London, W.C.1), at 7 p.m.—Mr. C. H. Gardner: "Germanium Transistors, Their Principles and Manufacture".

CHEMICAL SOCIETY (in the Anatomy Lecture Theatre, King's College, Strand, London, W.C.2), at 7.30 p.m.—Prof. R. G. W. Norrish, F.R.S.: "Some Isothermal Reactions of Free Radicals Studied by Kinetic Spectroscopy" (Liversidge Lecture).

Friday, February 28

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Mr. L. J. Cheshire, Mr. J. V. G. Evans, Mr. W. A. Goodsell and Mr. P. H. W. Wolff: "The Design and Construction of the Compressor for the 8′ × 8′ High-Speed Wind Tunnel at R.A.E., Bedford".

INSTITUTION OF CHEMICAL ENGINEERS, GRADUATES' AND STUDENTS' SECTION (at Caxton Hall, Westminster, London, S.W.1), at 6.30 p.m.—Mr. E. A. Stallworthy: "Project Cost Estimation".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. Leon Radzinowicz: "Changing Attitudes Towards Crime and the Devices Used to Combat It".

Saturday, March 1

BRITISH INTERPLANETARY SOCIETY (in the Tudor Room, Caxton Hall, Caxton Street, London, S.W.1), at 6 p.m.—Prof. G. A. Witfield: "The Guidance and Control of Long-Range Vehicles".

BRITISH GLACIOLOGICAL SOCIETY (joint meeting with the SCOTT POLAR RESEARCH INSTITUTE, at the Scott Polar Research Institute, Lensfield Road, Cambridge), at 8.30 p.m.—Mr. R. J. E. Brown: "Permafrost in Canada—Its Nature, Distribution and Influence on Northern Development".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT LECTURERS (2) (honours graduates, preferably with some research experience in physical, inorganic or physical-organic chemistry) IN CHEMISTRY—The Secretary, Bedford College (University of London), Regent's Park, London, N.W.1 (February 28).

PROFESSOR (with high academic qualifications in engineering with teaching and research or industrial experience) OF APPLIED MECHANICS at the Royal Naval College, Greenwich—The Civil Service Commission, 6 Burlington Gardens, London, W.1, quoting Ref. No. 4808/58 (March 4).

ASSISTANT LECTURER IN GEOGRAPHY; an ASSISTANT LECTURER IN GEOLOGY; and an ASSISTANT LECTURER IN PHYSICS—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1 (March 8).

ASSISTANT LECTURER or LECTURER IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, The University, Liverpool (March 8).

LECTURER IN GEOLOGY at the University College of Ghana—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 10).

UNIVERSITY LECTURER (preferably with qualifications in applied thermodynamics) IN MECHANICAL ENGINEERING—The Secretary, The University, Aberdeen (March 11).

ASSISTANT LECTURER (preferably with special qualifications in algebra and/or geometry) IN MATHEMATICS—The Secretary to the Council, Westfield College (University of London), Hampstead, London, N.W.3 (March 12).

ASSISTANT LECTURER IN NEAR EASTERN ARCHÆOLOGY—The Registrar, The University, Manchester 13 (March 15).

ASSISTANT LECTURER or LECTURER (with special interests in sedimentation) IN THE DEPARTMENT OF GEOLOGY—The Registrar, University College, Singleton Park, Swansea (March 15).

EXPERIMENTAL OFFICER (with a degree in chemistry or agricultural chemistry or equivalent qualifications), to carry out chemical analyses of soils and plants—The Secretary, Rothamsted Experimental Station, Harpenden, Herts (March 15).

PROFESSOR or READER IN AGRICULTURE—The Registrar, The University, Nottingham (March 15).

RESEARCH ASSISTANT (graduate, with research experience) IN THE DEPARTMENT OF PHYSICS, to take part in research, under the direction of the Head of the Department, leading to the development of solid state image amplifiers; and an ASSISTANT LECTURER IN GEOGRAPHY—The Registrar, The University, Hull (March 15).

LECTURER or ASSISTANT LECTURER IN MATHEMATICS at the University of Hong Kong—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Hong Kong, March 20).

ASSISTANT LECTURER or LECTURER IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, The University, Birmingham 15 (March 24).

SENIOR RESEARCH FELLOW and RESEARCH FELLOW IN THE DEPARTMENT OF THEORETICAL PHYSICS, Research School of Physical Sciences, Australian National University—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, March 28).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY, BIOLOGICAL CHEMISTRY, ENGINEERING, PHARMACOLOGY, PHYSICS or related subjects—The Registrar, The University, Bristol (March 30).

LECTURER and an ASSISTANT LECTURER IN PHYSICS—The Registrar, The University, Hull (March 31).

LECTURER or ASSISTANT LECTURER IN AGRICULTURAL BOTANY at Makerere College (University College of East Africa)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (March 31).

PLANT GENETICIST (preferably with some research experience) IN THE PLANT BREEDING SECTION of the Department of Agronomy, Waite Agricultural Research Institute, to investigate factors affecting the quality and yield in barley and their mode of inheritance and to apply them in a barley breeding programme—The Registrar, University of Adelaide, Adelaide, South Australia (March 31).

RESEARCH FELLOW (zoologist, with interests in marine biology) IN THE DEPARTMENT OF ZOOLOGY—The Registrar, University College, Singleton Park, Swansea (March 31).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOW IN CHEMISTRY OR PHYSICS—The Registrar, The University, Leicester (April 9).

ASSISTANT or ASSOCIATE PROFESSOR IN THE DEPARTMENT OF ENTOMOLOGY—Head of the Department of Entomology, University of Alberta, Edmonton, Alberta, Canada (April 15).

CHAIR OF PHYSICS in the University of Khartoum—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 15).

SENIOR LECTURER or LECTURER IN EPIZOOTIOLOGY in the Faculty of Veterinary Science, University of Khartoum—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 15).

SENIOR LECTURERS or LECTURERS IN MATHEMATICS, GEOLOGY, ZOOLOGY, AGRICULTURAL CHEMISTRY, ENGINEERING MATHEMATICS, MECHANICAL ENGINEERING, and ARCHITECTURE at the University of Khartoum—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (April 15).

SENIOR LECTURER (preferably with special qualifications in numerical analysis) IN MATHEMATICS at Victoria University, Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, April 30).

ASSISTANT CONSERVATORS OF FORESTS (with a university degree in forestry) in the Western Region of Nigeria—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.61/410/01.

ASSISTANT PROFESSOR (preferably with the Ph.D. degree or equivalent) IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Department of Mechanical Engineering, University of Vermont, Burlington, Vermont, U.S.A.

HEAD (with qualifications, teaching and research experience necessary for recognition as a teacher of the University of London) OF THE DEPARTMENT OF METALLURGY—The Secretary, Sir John Cass College, Jewry Street, London, E.C.3.

LECTURER (preferably with experience and interest in experimental techniques) IN AERONAUTICAL STRUCTURES—Professor of Aeronautical Structures, Department of Aeronautics, Imperial College of Science and Technology, London, S.W.7.

SCIENTIFIC OFFICER (with an honours degree in biochemistry, chemistry or physiology, and preferably with postgraduate experience) WITH THE EAST AFRICAN TRYPANOSOMIASIS RESEARCH ORGANIZATION, Tororo, Uganda, to undertake research on the physiological, biochemical and/or chemical aspects of trypanosomiasis related especially to the assessment of the efficacy and toxicity of new trypanocidal drugs in East African cattle—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.195/198/013.

SENIOR LECTURER (honours graduate, preferably with research and/or industrial experience) IN PHYSICS—The Registrar, Hatfield Technical College, Hatfield, Herts.

SYSTEMATIC BOTANIST AND ECOLOGIST (with an honours degree in botany and at least two years research experience, preferably in ecology; or an honours degree or equivalent in agricultural science plus two years completed postgraduate training in botany, preferably with an ecological bias) in Sierra Leone, to carry out ecological studies appertaining to agricultural and forestry problems (including the maintenance and enlargement of a herbarium)—The Director of Recruitment, Colonial Office, London, S.W.1, quoting BCD.63/15/012.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Ministry of Health: Department of Health for Scotland. Report of the Committee to Consider the Future Numbers of Medical Practitioners and the Appropriate Intake of Medical Students. Pp. vi+38. (London: H.M. Stationery Office, 1957.) 2s. 6d. net. [212

Department of Scientific and Industrial Research. Forest Products Research. A Handbook of Softwoods. Pp. vii+73. (London: H.M. Stationery Office, 1957.) 8s. 6d. net. [212

Structural Concrete Hospitals. Pp. 24. (London: Cement and Concrete Association, 1957.) [212

Science Masters' Association. Science and Education: a Policy Statement by the Committee of the Science Masters' Association. Pp. 12. (London: John Murray (Publishers), Ltd., 1957. Published for the Science Masters' Association.) [212

Trade Effluent and Water Supplies in Western Germany: Report of a tour by an F.B.I. Delegation. Pp. iv+64. (London: Federation of British Industries, 1957.) 3s. 6d. [212

Laboratory Animals Bureau. Collected Papers, Vol. 6, 1957: Report of a Symposium on Humane Technique in the Laboratory, held by the Universities Federation for Animal Welfare at Birkbeck College, London, 8 May, 1957. Pp. 81. (London: Laboratory Animals Bureau, 1957.) 10s. 212

Boiler Availability Committee. Bulletin No. MC/286: Methods of Cleaning Modern Boiler Plant. Pp. 40. (London: Boiler Availability Committee, 1957.) [212

Philosophical Transactions of the Royal Society of London. Series A: Mathematical and Physical Sciences. No. 978, Vol. 250 (28 November, 1957): Precise Measurements of the Density of Mercury at 20° C. 1: Absolute Displacement Method. By A. H. Cook and N. W. B. Stone. Pp. 279–323. (London: Royal Society, 1957.) 17s. [212

Local Government Bill. Pp. 78. (Bill 15.) (London: H.M. Stationery Office, 1957.) 3s. 6d. [212

Fifth Special Report from the Select Committee on Estimates, Session, 1956–57. Pp. 25. (House of Commons Paper, No. 307.) (London: H.M. Stationery Office, 1957.) 1s. 3d. net. [212

Colonial Office. Fishery Publications, No. 9 (1957): A Preliminary Survey of the Hydrography of the British East African Coastal Waters. By B. S. Newell. Pp. ii+21. (London: H.M. Stationery Office, 1957.) 5s. 6d. net. [412

City of Leicester Museums and Art Gallery. 51st Report to the City Council, 1st April 1956–31st March, 1957. Pp. 43+4 plates. (Leicester: The Museums and Art Gallery, 1957.) [612

The Planning of International Meetings. (A Handbook issued by the Council for International Organizations of Medical Sciences established under the joint auspices of UNESCO and WHO.) Pp. v+113. (Oxford: Blackwell Scientific Publications, 1957.) 7s. 6d. [612

Southern Regional Council for Further Education. Tenth Annual Report, October 1957. Pp. 36. (Reading: Southern Regional Council for Further Education, 1957.) [612

The National Council for Technological Awards. Report for the period December 1955 to July 1957. Pp. 20. (London: National Council for Technological Awards, 1957.) [612

Air Ministry: Meteorological Office. Professional Notes, No. 123: Forecasting Cirrus Cloud Over the British Isles. By Dr. D. G. James. Pp. 10 (M.O. 623c.) 9d. net. Meteorological Reports, No. 18: Air Flow Over Mountains: Notes for Forecasters and Pilots. G. A. Corby. Pp. ii+53. 3s. net. (London: H.M. Stationery Office, 1957.) [612

Pharmaceutical Research in I.C.I., 1936–57. Pp. viii+78. (Wilmslow: Imperial Chemical Industries, Ltd., 1957.) [1612

Other Countries

United States Department of the Interior: Geological Survey Water-Supply Paper 1305: Compilation of Records of Surface Water of the United States through September 1950. Part 3–A: Ohio River Basin except Cumberland and Tennessee River Basins. Prepared under the direction of J. V. B. Wells. Pp. v+652+plate 1. 2.25 dollars. Water-Supply Paper 1331: Surface Water Supply of the United States, 1954. Part 1–A: North Atlantic Slope Basins, Maine to Connecticut. Prepared under the direction of J. V. B. Wells. Pp. viii+261. 1 dollar. Water-Supply Paper 1372: Compilation of Records of Quantity and Quality of Surface Waters of Alaska through September 1950. Prepared under the direction of J. V. B. Wells and S. K. Love. Pp. v+262+1 plate. 1.50 dollars. Water-Supply Paper 1384: Surface Water Supply of the United States, 1955. Part 2–B: South Atlantic Slope and Eastern Gulf of Mexico Basins, Ogeechee River to Pearl River. Prepared under the direction of J. V. B. Wells. Pp. x+388. 1.25 dollars. Water-Supply Paper 1385: Surface Water Supply of the United States, 1955. Part 3–A: Ohio River Basin except Cumberland and Tennessee River Basins. Prepared under the direction of J. V. B. Wells. Pp. xii+581. 2 dollars. (Washington, D.C.: Government Printing Office, 1957.) [2511

Forest Research Institute, Dehra Dun. Indian Forest Leaflet No. 147 (Timber Mechanics): Strength Tests on Bamboos. By A. C. Sekhar and B. S. Rawat. Pp. 6. 0.56 nP; 10d. Indian Forest Leaflet No. 153 (Statistical): Statistical Sampling in Timber Surveys. By K. R. Nair and R. P. Bhargava. Pp. 1+7. 0.75nP.; 1s. Indian Forest Records (New Series.) Vol. 1, No. 1 (Wood Seasoning): The Steam Bending Properties of Indian Timbers. By M. A. Rehman, Jai Kishen and D. P. Kukreti. Pp. ii+10. Re. 1; 1s. 6d. Vol. 1, No. 2 (Timber Mechanics): Grouping of Indian Timbers and their Properties, Uses and Suitability. By V. D. Limaye. Pp. 19–64+24 plates. Rs. 9.50; 15s. (Delhi: Manager of Publications, 1957.) [212

The Structure and Reproduction of some Members of the Rhodymeniaceae. By Shirley Ray Sparling. (University of California Publications in Botany, Vol. 29, No. 3.) Pp. 319–396. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1957.) 1.50 dollars. [212

International Sanitary Regulations adopted by the Fourth World Health Assembly in 1951, and amended by the Eighth and Ninth World Health Assemblies in 1955 and 1956. Annotated edition. Pp. 129. (Geneva: World Health Organization; London: H.M. Stationery Office, 1957.) 3 Swiss francs; 5s.; 1 dollar. [212

Norsk Polarinstitutt. Meddelelser Nr. 80: Research Into the Prevalence of Trichinosis in Animals in Arctic and Antarctic Waters. By K. Thorshaug and A. Fr. Rosted. Pp. 115–130. 1.50 Kr. Meddelelser Nr. 81: Glacier Dammed Lakes in Norway. By Olav Liestøl. Pp. 121–150. 2 Kr. Skrifter, Nr. 109: The Quaternary Geology of Bragenesset, Nordaustlandet, Spitsbergen. By J. J. Donner and R. G. West. Pp. 29+5 plates. 5 Kr. Skrifter, Nr. 110: Magnetic Observations in Svalbard, 1596–1953. By Kaare Z. Lundqvist. Pp. 34. 6 Kr. Skrifter, Nr. 111: The Stress of the Wind on the Ice of the Polar Sea. By H. U. Sverdrup. Pp. 11. 2 Kr. (Oslo: Norsk Polarinstitutt, 1956 and 1957.) [212

Fortieth Annual Report of the National Research Council of Canada, 1956–57, including the Annual Report of Canadian Patents and Development Limited. Pp. 58. (N.R.C. 4370.) (Ottawa: Queen's Printer, 1957.) [212

English Version of Introduction and Remarks from the Seventh Supplement to the First Edition of the General Catalogue of Variable Stars (by B. V. Kukarkin, P. P. Parenago, Yu I. Efremov and P. N. Kholopov, Moscow, 1955); Containing Information about 261 Variable Stars, which were newly named during 1955, and also more precise information about 358 previously named Variable Stars; and from the Eighth Supplement to the First Edition of the General Catalogue of Variable Stars (by B. V. Kukarkin, P. P. Parenago, Yu I. Efremov and P. N. Kholopov, Moscow, 1956); containing information about 629 Variable Stars which were newly named during 1956. Pp. 52. (Leiden: International Astronomical Union, 1957.) [212

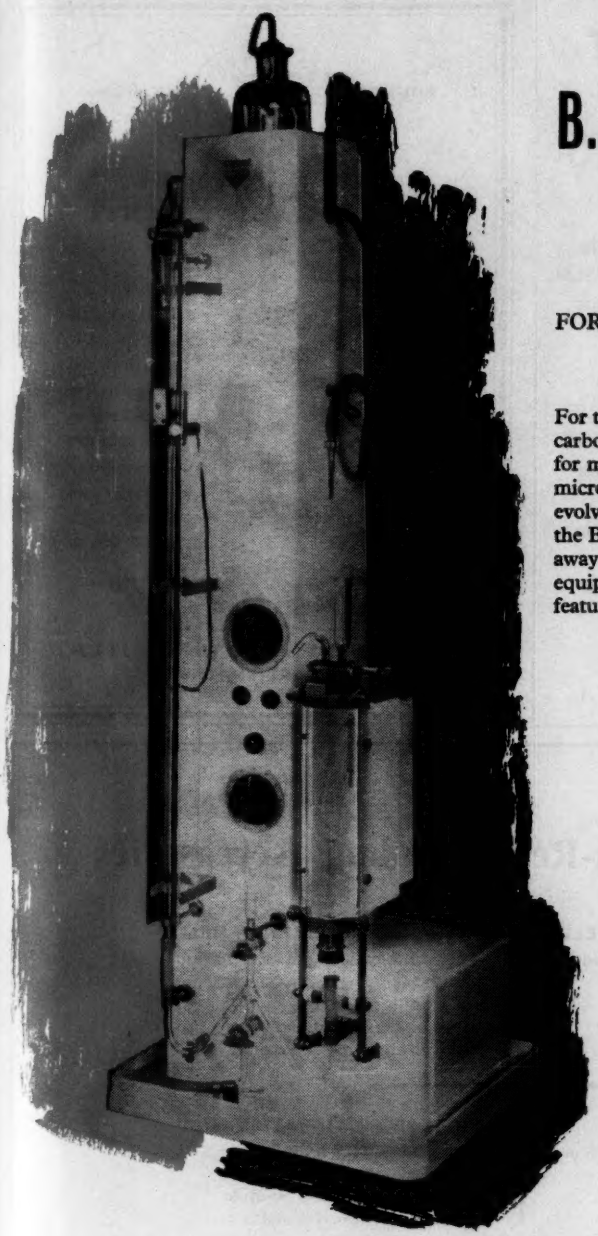
Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture. Comptes Rendus de Recherches, Nr. 19: Travaux du Centre National de Recherches Métallurgiques. Pp. 122. (Bruxelles: Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture, 1957.) 130 Belgian francs. [212

Editorial and Publishing Offices of "NATURE"
MACMILLAN & CO., LTD.,
ST. MARTIN'S STREET, LONDON, W.C.2.
Telephone Number: Whitehall 8831. Telegrams: Phusis Lesquare London

Annual subscription £7/15/–, payable in advance, postage paid to any part of the world

Advertisements only should be addressed to
T. G. Scott & Son, Ltd., 1 Clement's Inn, London, W.C.2
Telephone Number: Holborn 4743

All rights reserved. Registered as a newspaper at the General Post Office



B.T.L. Van Slyke Apparatus

FOR STANDARD MANOMETRIC GAS ANALYSIS

For the determination of carbon dioxide or carbon monoxide in blood, plasma etc. and for many applications in biochemistry and micro-analysis where an amount of gas evolved from a fluid has to be measured, the B.T.L. Van Slyke Apparatus is far and away the most advanced and convenient equipment now available. The principal features are:

- Shaking motor with variable speed control up to 200 per minute.
- The fine capillaries employed give extremely accurate control of the mercury.
- Graduated manometer with illuminated meniscus reader.
- Shaking pipette, with sloping shoulders to avoid trapping gas bubbles.
- Glass water-jacket, with thermometer, illuminated from rear so that pipette is clearly seen.
- Synchronous motor-operated timer and alarm buzzer can be fitted as optional extra.

BTL

Write for Technical Publication T.64

complete laboratory service

BAIRD & TATLOCK (LONDON) LTD., CHADWELL HEATH, ESSEX, ENGLAND.

Branches in London, Manchester, Glasgow. *Agents throughout U.K. and all over the world.*

TAS/BT. 19

PHASE CONTRAST

This method supplements the more usual techniques of microscopical illumination, rendering visible details not observable by other methods.

Full particulars on application

R. & J. BECK LTD.
69/71 Mortimer Street, London, W.1

FOUNDED HAWKSLEY LONDON IN 1869

THE HAWKSLEY MICRO-HAEMATOCRIT CENTRIFUGE

A new, high-speed centrifuge specially designed to give complete cell packing in five minutes using a micro quantity of capillary or venous blood.

Your usual supplier of Hawksley products will gladly give full details

HAWKSLEY & SONS LIMITED
LONDON, ENGLAND

Ultra-Violet & Infra-Red Sampling Accessories

ULTRA-VIOLET ABSORPTION CELLS
VARIABLE path-length cells for liquids
FIXED path-length cells for liquids

KBR EVACUABLE DIE
for making 13, 15 and 16 mm. Discs

INFRA-RED ABSORPTION CELLS
VARIABLE path-length cells for liquids
FIXED path-length demountable and sealed cells for liquids
MICROCELLS for liquids
GAS cells in pyrex and metal

HYDRAULIC 20-TON PRESS
especially designed for KBr Disc Technique

Rotating Electrode (Solution Excitation) Spark Unit

HIGH-SPEED VIBRATION MILL
indispensable for KBr Disc Technique
available for 230 volt 50-cycle and 110-volt 60-cycle mains

RII Sampling Accessories are marketed by the leading spectrometer manufacturers of the world. Descriptive literature on application.

RESEARCH & INDUSTRIAL INSTRUMENTS CO.
30 Langton Road, London SW9 Telephone : RELiance 6171

Printed in Great Britain by FISHER, KNIGHT & CO., LTD., The Gainsborough Press, St. Albans, and published by MACMILLAN & CO., LIMITED, at St. Martin's Street, London, W.C.2, and ST. MARTIN'S PRESS, INC., 103 Park Avenue, New York 17, N.Y., U.S.A.—Saturday, February 22, 1958.